Universidade Federal de Minas Gerais

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Departamento de Morfologia

Tese de Doutorado

EFEITOS DA CONTAMINAÇÃO POR METAIS PESADOS E BIOMARCADORES DE IMPACTO AMBIENTAL EM PEIXES DA BACIA DO RIO SÃO FRANCISCO, MG

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Universidade Federal de Minas Gerais

Setembro/2019

LOURENÇO ALMEIDA SAVASSI

EFEITOS DA CONTAMINAÇÃO POR METAIS PESADOS E BIOMARCADORES DE IMPACTO AMBIENTAL EM PEIXES DA BACIA DO RIO SÃO FRANCISCO, MG

Tese apresentada ao Programa de Pós-Graduação em Biologia Celular do Departamento de Morfologia, do Instituto de Ciências Biológicas, da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do título de Doutor em Ciências.

Área de concentração: Biologia Celular

Orientador: Dr. Nilo Bazzoli
Co-orientadora: Drª. Elizete Rizzo

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Às **oito horas** do dia **30 de setembro de 2019**, reuniu-se, no Instituto de Ciências Biológicas da UFMG, a Comissão Examinadora da Tese, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho final intitulado: **"EFEITOS DA CONTAMINAÇÃO POR METAIS PESADOS E BIOMARCADORES DE IMPACTO AMBIENTAL EM PEIXES DA BACIA DO RIO SÃO FRANCISCO, MG"**, requisito final para obtenção do grau de Doutor em Biologia Celular. Abrindo a sessão, o Presidente da Comissão, **Dr. Nilo Bazzoli**, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra ao candidato, para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do candidato. Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	Instituição	Indicação
Dr. Nilo Bazzoli	UFMG	APROVADO
Dr. José Enemir dos Santos	PUC MINAS	APROVADO
Dr. Lucas Marcon	UEMG	APROVADO
Dr. Guilherme Mattos Jardim Costa	UFMG	APROVADO
Dra. Gleide Fernandes de Avelar	UFMG	APROVADO

Pelas indicações, o candidato foi considerado: ______________________________

O resultado final foi comunicado publicamente ao candidato pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por todos os membros participantes da Comissão Examinadora. **Belo Horizonte, 30 de setembro de 2019.**

Dr. Nilo Bazzoli (Orientador) ______________________________

Dr. José Enemir dos Santos ______________________________

Dr. Lucas Marcon ______________________________

Dr. Guilherme Mattos Jardim Costa ______________________________

Drª. Gleide Fernandes de Avelar ______________________________

Prof. Vanessa Pinho da Silva
Sub-Coordenadora do Programa de
Pós Graduação em Biologia Celular ICB/UFMG

Obs: Este documento não terá validade sem a assinatura e carimbo do Coordenador

Vanessa Pinho da Silva

O presente trabalho foi desenvolvido no laboratório de Ictiohistologia, do Departamento de Morfologia, do Programa de Pós-Graduação em Biologia Celular, Instituto de Ciências Biológicas, UFMG, coordenado pela Profª. Drª. Elizete Rizzo, para desenvolvimento geral do projeto, adicionalmente incluindo todo o apoio técnico disponibilizado pelo laboratório de Ictiologia, do Programa de Pós-Graduação em Biologia de Vertebrados, PUC Minas, coordenado pelo Prof. Dr. Nilo Bazzoli.

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Dedico este trabalho à toda minha família, especialmente meus pais Marcelo e Simone e minha esposa Alessandra.

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SUMÁRIO

RESUMO

Metais pesados são elementos inorgânicos que podem provocar sérios danos à saúde dos organismos vivos em geral. Através de resíduos industriais, rurais e domésticos, metais como Cádmio (Cd), Chumbo (Pb), Cobre (Cu), Cromo (Cr), Ferro (Fe) e Zinco (Zn), atingem constantemente os ambientes aquáticos. Esses poluentes geralmente não são degradados naturalmente e possuem alta capacidade de bioacumulação em tecidos e órgãos gerando graves patologias. Diante desse problema ambiental o objetivo do presente estudo foi avaliar, através de análises histológicas e imunohistoquímicas, o estado de contaminação do dourado, *Salminus franciscanus*, espécie de peixe de grande porte, endêmico da bacia do rio São Francisco, MG, que representa alto valor comercial e é amplamente consumido na alimentação da população. Foram avaliados os níveis de Cd, Cr, Cu, Fe, Pb e Zn, no fígado e no tecido muscular, de 68 exemplares de *S. franciscanus* coletados em dois rios da bacia do rio São Francisco, MG, Brasil: Abaeté (pouca atividade antrópica) e Paraopeba, trechos A e B (alta atividade antrópica). Em conjunto foram avaliadas alterações histopatológicas no fígado e baço (órgãos de intenso metabolismo), e expressão de três proteínas consideradas atualmente biomarcadoras de impacto ambiental: Metalotioneína (MT), Proteína de Choque Térmico-70 (HSP70) e Citocromo P450-1A (CYP1A). Os resultados mostraram que os peixes do rio Paraopeba estão impróprios ao consumo humano, ultrapassando os limites seguros ao consumo no filé (Cd, Cr, Cu e Pb acima do limite permitido) estabelecidos pela Organização Mundial de Saúde (OMS) e pela Agência Nacional de Vigilância Sanitária (ANVISA). Além dos elevados níveis de metais pesados na musculatura dos peixes, no fígado foram registrados valores acima do limite legal para os metais Cd, Cr, Cu, Fe, Pb e Zn (todos os metais analisados) sendo registradas e quantificadas alterações histopatológicas no fígado e baço dos peixes, indicando diferenças significativas à pior qualidade ambiental e ao cenário de maior contaminação por metais pesados, encontrado no rio Paraopeba. Também foram registradas diferenças significativas nos níveis de expressão das proteínas MT, HSP70 e CYP1A, entre os rios Abaeté e Paraopeba, evidenciando a alta contaminação em ambos os trechos de amostragem no rio Paraopeba em relação ao rio Abaeté.

Palavras-chave: Bioindicadores; Poluição aquática; Toxicologia; Teleósteos.

ABSTRACT

Heavy metals are inorganic elements that can cause serious health problems in living organisms in general. Through industrial, rural and domestic waste, metals such as Cadmium (Cd), Lead (Pb), Copper (Cu), Chromium (Cr), Iron (Fe) and Zinc (Zn) constantly reach aquatic environments. These pollutants are usually not naturally degraded and have a high capacity of bioaccumulation in tissues and organs inducing pathologies. Due this environmental problem, the objective of the present study was to evaluate, through histological and immunohistochemical analyzes, the condition of contamination of dourado, *Salminus franciscanus*, a large fish species, endemic to the São Francisco River Basin, MG, which represents a high commercial value and is widely consumed in population feeding. Levels of Cd, Cr, Cu, Fe, Pb and Zn was quantified in the liver and muscle tissue, of 68 specimens of *S. franciscanus* collected from two rivers of the São Francisco River Basin, MG, Brazil: Abaeté (low anthropic activity) and Paraopeba, sites A and B (high anthropic activity). Histopathological alterations in the liver and spleen (organs of intense metabolism) and expression of three proteins currently considered biomarkers of environmental impact, were evaluated: Metallothionein (MT), Heat Shock Protein-70 (HSP70) and Cytochrome P450-1A (CYP1A). The results evidenced that fish from the Paraopeba River are inappropriate for human consumption, exceeding the safe limits for muscle consumption (Cd, Cr, Cu and Pb above the permitted limit) established by the World Health Organization (WHO) and the Agência Nacional de Vigilância Sanitária (ANVISA). In addition to the high levels of heavy metals registered in fish muscles, in liver the values were recorded above the legal limit for Cd, Cr, Cu, Fe, Pb and Zn metals (all metals analyzed) and histopathological alterations in liver and spleen were recorded and quantified, indicating significant differences in the poor environmental quality and the scenario of high contamination by heavy metals found in Paraopeba River. Significant differences in expression levels of MT, HSP70 and CYP1A proteins, were also recorded between the Abaeté and Paraopeba Rivers, evidencing the high contamination in both sampling sites of Paraopeba River in relation to Abaeté River.

Keywords: Bioindicators; Teleosts; Toxicology; Water pollution.

LISTA DE FIGURAS

1. INTRODUÇÃO GERAL

O crescimento mundial da população provocou a expansão de atividades urbanas e rurais, desencadeando um aumento nas atividades industriais e na produção de resíduos químicos, que atualmente são produzidos em larga escala, constituindo fontes poluidoras dos recursos hídricos, fauna e flora (Cao et al., 2017; Kwok et al., 2014; Jiang et al., 2018). A poluição dos corpos d'água implica em substâncias tóxicas que afetam a saúde dos peixes e consequentemente da população que utiliza essa carne na alimentação (Barone et al., 2013; Yi et al., 2017; Zhong et al., 2018).

O aumento da contaminação aquática por metais pesados, provenientes de atividades antrópicas como minerações, agropecuária e indústrias, atualmente vem se tornando um problema ambiental cada vez mais reportado em estudos toxicológicos (Ribeiro et al., 2012, Squadrone et al., 2013). Metais pesados são persistentes e não se degradam, acumulando-se nos tecidos de peixes e outros organismos aquáticos (Marques et al., 2009; D'Costa et al., 2017; Lunardelli et al., 2018). Neste sentido, a capacidade dos peixes em assimilar compostos inorgânicos no organismo, os tornam importantes bioindicadores ambientais (Gomes & Sato, 2011; Tabinda et al., 2013; Luczynska et al., 2018). A capacidade bioacumulativa dos peixes os tornam interessantes modelos de estudo para eventos da biologia celular, tais como a expressão de proteínas bioindicadoras de impacto ambiental, assim como ferramentas ecológicas para uma avaliação da qualidade do meio ambiente refletida no organismo vivo (De la Torre et al., 2007; Thomé et al., 2009, Monferran et al., 2016).

A carne dos peixes para a alimentação humana é reconhecida como excelente fonte de vitaminas, proteínas e minerais, porém se torna necessária uma eficaz avaliação das concentrações de metais pesados e outros contaminantes na carne, para um consumo seguro da população (Souza, 2003; Reis et al., 2009; Taweel et al., 2013). Nos peixes, os metais pesados podem causar distúrbios no crescimento e na reprodução, além de alterações histopatológicas na pele, brânquias, fígado, baço e rins (Vitek et al., 2007). O fígado e o baço participam do metabolismo e detoxificação de substâncias no organismo, e o tecido muscular, amplamente utilizado na alimentação da população, sendo assim, ambos devem ser analisados em relação ao nível de contaminação antes do consumo humano (Mendil et al., 2005; Castro-González & Méndez-Armenta, 2008; Subotić et al., 2013).

Para documentar e quantificar os efeitos de poluentes nos ambientes aquáticos, alguns biomarcadores fisiológicos e histopatológicos de peixes vêm sendo utilizados, tais como: apoptose, proteínas do choque térmico, metalotioneínas, alterações hormonais e reprodutivas, além de alterações histopatológicas em órgãos alvo, tais como: baço, brânquias, fígado e gônadas (Santos et al., 2005; Fishelson, 2006; Thomé et al., 2009; Prado et al., 2011; Arantes et al., 2016; Savassi et al., 2016; Paschoalini et al., 2019). A utilização desses biomarcadores de impacto ambiental em conjunto às análises de metais pesados, fornecem informações cruciais para o entendimento da influência de metais pesados sobre o organismo, assim como ajudar agências governamentais a criar atualizados limites de contaminantes, na carne dos peixes.

1.1 Metais pesados

Metais pesados são definidos como elementos metálicos que apresentam elevada massa e número atômico, alta densidade, capacidade de formar sulfetos e hidróxidos insolúveis em água, serem de fácil absorção por um organismo vivo, além de alta capacidade tóxica em baixas concentrações (Fergusson, 1990; Tchounwou et al., 2012). Nos últimos anos, tem surgido uma crescente preocupação ecológica, e de saúde pública global, associada à contaminação ambiental por metais pesados. Devido ao aumento exponencial do uso desses metais em várias aplicações industriais, agrícolas, domésticas e tecnológicas, tem sido reportado em estudos científicos um aumento da exposição humana a esses elementos (Jia et al., 2018; Hossain et al., 2018; Luczyska et al., 2018).

Estudos recentes apontam metais pesados amplamente distribuídos nos ambientes aquáticos (Figura 1), dentre eles Arsênio (As), Cádmio (Cd), Chumbo (Pb), Cobre (Cu), Mercúrio (Hg) e Zinco (Zn) que são altamente bioacumulativos e tendem a aumentar a concentração no organismo dos animais com o passar dos anos, pois não conseguem ser eliminados fisiologicamente (Squadrone et al., 2013; Protano et al., 2014). O Cromo (Cr) apesar de não indicar ser bioacumulativo, tem efeitos tóxicos sobre o organismo, assim como os outros elementos (Arantes et al., 2016; Masindi & Muedi, 2018). Esse acúmulo de compostos tóxicos no organismo provoca a formação de patologias em órgãos alvos, como a esteatose no fígado, fibrose no baço, lesões na porção cranial dos rins, necroses e câncer em diversos órgãos (Vieira et al., 2011; Thévenod & Lee, 2015; Paschoalini et al., 2019). Em concentrações elevadas, estes metais também podem interromper a homeostase de íons, induzir a danos no DNA e distúrbios em vários órgãos (Vieira et al., 2011; Nai et al., 2015; Li et al., 2015), afetando organelas celulares e componentes como a membrana celular, mitocôndria, lisossomo, retículo endoplasmático, núcleo e enzimas envolvidas no metabolismo, desintoxicação e reparo de danos no organismo (Wang & Shi, 2001).

Intensificando a contaminação por metais pesados através da bioacumulação, quando um animal de cadeia alimentar superior se alimenta de um animal posicionado em posição inferior contaminado, ocorre a propagação da contaminação através da cadeia alimentar, fenômeno denominado de Biomagnificação (Suedel et al., 1994). Esse processo intensifica a contaminação pois os metais pesados têm alto poder bioacumulativo, aumentando sua concentração no organismo com o passar dos anos e consequentemente elevando seu potencial tóxico (Ali & Khan, 2018; Liu et al., 2019).

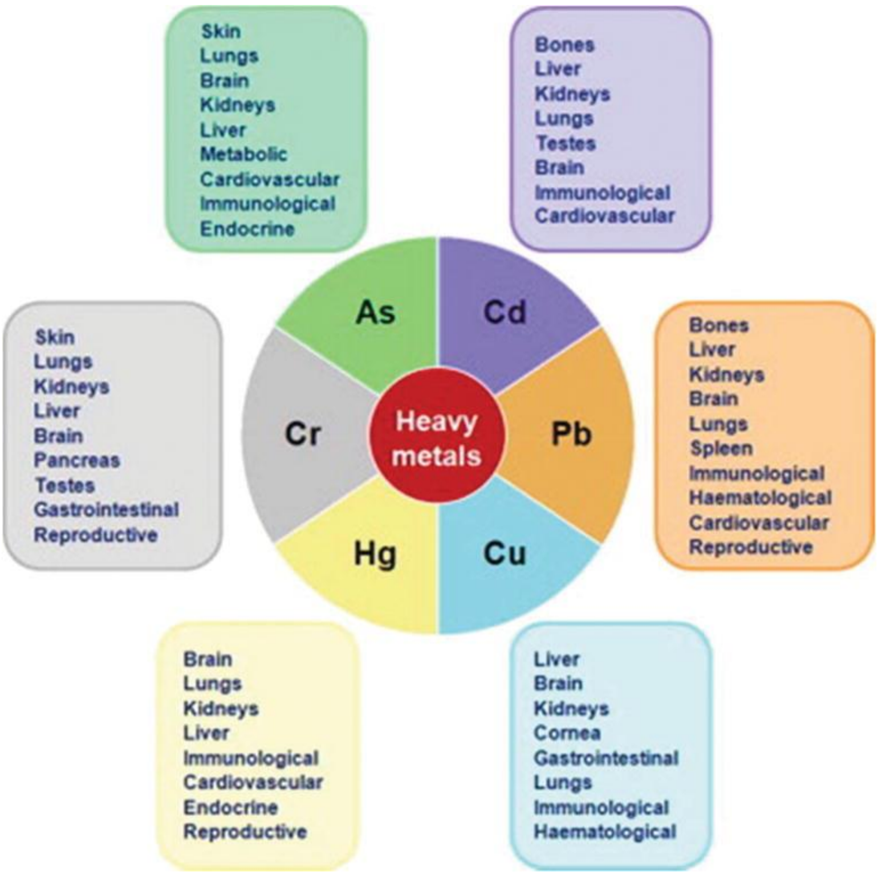

Figura 1. Sistemas e órgãos afetados pelos diferentes tipos de metais pesados, no organismo. Reproduzido de Masindi & Muedi, 2018.

1.2 Metalotioneínas

Metalotioneínas (MT) são uma classe de proteínas citosólicas, ricas em cisteínas, caracterizadas pelo baixo peso molecular e afinidade por cátions divalentes. As funções biológicas das MT envolvem desintoxicação e homeostase de íons metálicos, sendo importantes no metabolismo intracelular de cobre (Cu), zinco (Zn) e cádmio (Cd), atuando na proteção contra o dano oxidativo e toxicidade resultante da exposição excessiva a metais-traço (Stillman, 1995; Sevcikova et al., 2013). Ainda se torna necessário elucidar o tipo de resposta de cada metal sobre os níveis de MT no organismo. A exposição crônica a metais pesados aumenta a produção de espécies reativas de oxigênio (ROS), como o peróxido de hidrogênio (H_2O_2), radical superóxido (O_2^-) e radical hidroxila (OH^-), podendo levar ao estresse oxidativo e causar peroxidação lipídica, danos no DNA e oxidar moléculas em membranas biológicas e tecidos, assim como demonstrado na Figura 2 (Atli & Canli, 2010; Mittler, 2002; Ruttkay-Nedecky et al., 2013). Órgãos relacionados ao intenso metabolismo e desintoxicação, como fígado e baço, são conhecidos locais de acúmulo de metais pesados e por isso devem ser direcionados a estudos que analisam a expressão de MT nesses órgãos de peixes teleósteos (Carvalho et al., 2004; Marijic & Raspor, 2006).

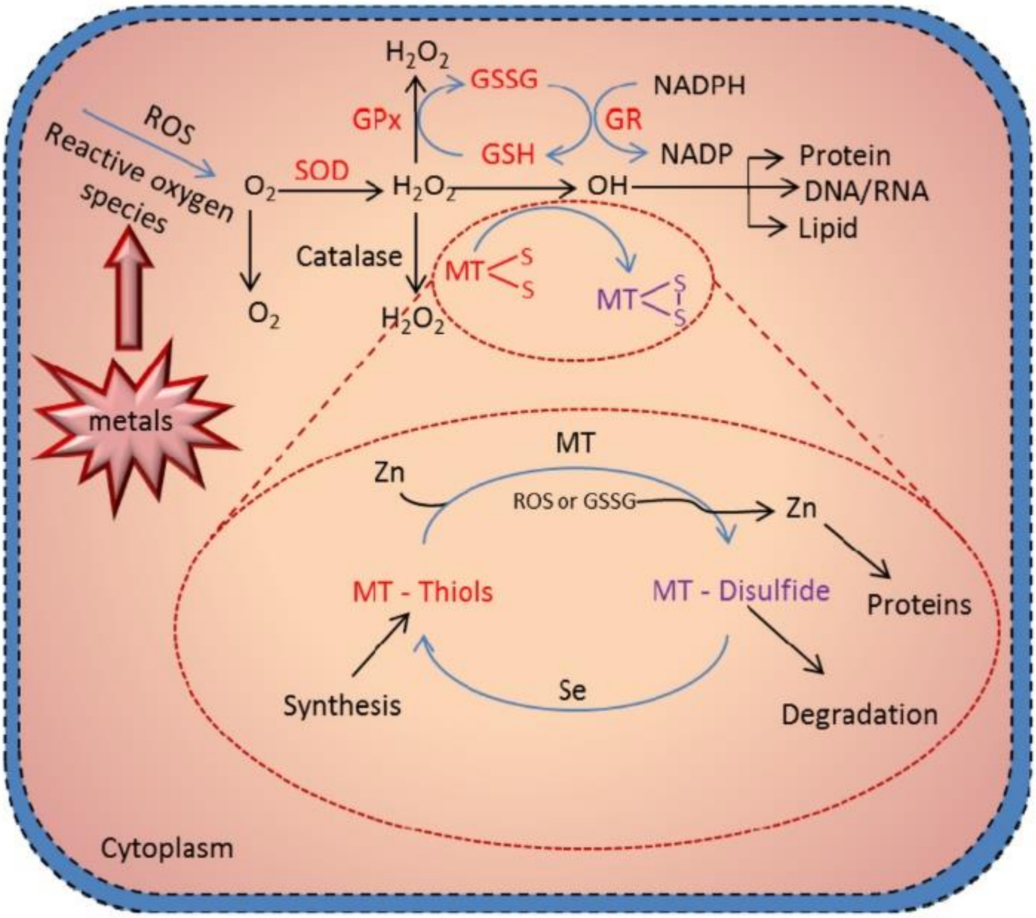

Figura 2. Desenho esquemático sobre a capacidade da MT como eliminadora de radicais livres e à indução a espécies reativas de oxigênio (ROS) nas células, causadas pelos metais pesados (Ruttkay-Nedecky et al., 2013).

Nos organismos aquáticos as concentrações de MT nos tecidos aumentam rapidamente, quando induzidos por exposição a metais-traço, como Cd, Cu, Zn e As, tanto em experimentos de laboratório quanto na natureza (Viarengo et al., 1999). Devido a sua especificidade e capacidade de se ligar a íons metálicos, a MT torna-se um excelente biomarcador, utilizado no monitoramento de ambientes aquáticos contaminados por metais-traço (Chan, 1995; El-Shehawi et al., 2007; Knapen et al., 2007; Rajeshkumar et al., 2013). Apesar de ser muito recomendada em estudos de contaminação por metais pesados, por confirmar a contaminação por metais pesados no organismo, a utilização de metalotioneínas em estudos toxicológicos, principalmente brasileiros, ainda é pouco estabelecida e necessita ser melhor investigada na ictiofauna.

1.3 Proteínas do choque térmico

Proteínas do choque térmico (Heat Shock Proteins) são altamente conservadas durante a evolução e funcionam como chaperonas moleculares que interferem na síntese proteica, promovendo a correta organização molecular de proteínas que se formaram de maneira incorreta (Feder & Hofmann, 1999; Parcellier et al., 2003). As HSP podem pertencer a diversas famílias e dentre elas a HSP70 é conhecida por interagir diretamente com elementos da via apoptótica, seja ela intrínseca ou extrínseca, inibindo a cascata de eventos que culminam com a morte celular (Mosser et al., 1997, Parcellier et al., 2003). Dentre os genes da família de resposta ao choque térmico, a HSP70 é um dos genes altamente conservados e o primeiro a ser induzido em resposta a diversos fatores estressantes gerados pelo ambiente sobre o organismo (Mukhopadhyay et al., 2003).

Estudos realizados em peixes teleósteos têm mostrado um aumento da expressão de HSP70, em locais onde a qualidade ambiental não é favorável à saúde dos peixes, devido a presença de poluentes ambientais, como metais pesados, sugerindo que o aumento destas proteínas está associado com a proteção celular, incluindo o aumento da apoptose em peixes submetidos experimentalmente a agentes tóxicos (Janz et al., 2001; Weber & Janz, 2001; Rajeshkumar et al., 2013). Sugere-se também, que metais pesados se acumulam nas células vivas e podem interagir com o grupo tiol das proteínas, influenciando na formação de proteínas anormais, as quais sinalizam para a indução de genes HSP70 (Figura 3) (Somasundaram et al., 2018). Estudos recentes apresentam resultados em que pode ocorrer um aumento na expressão da proteína HSP70 em casos de contaminação por metais pesados, porém essa relação ainda não é bem elucidada (Rajeshkumar et al., 2013; 2017).

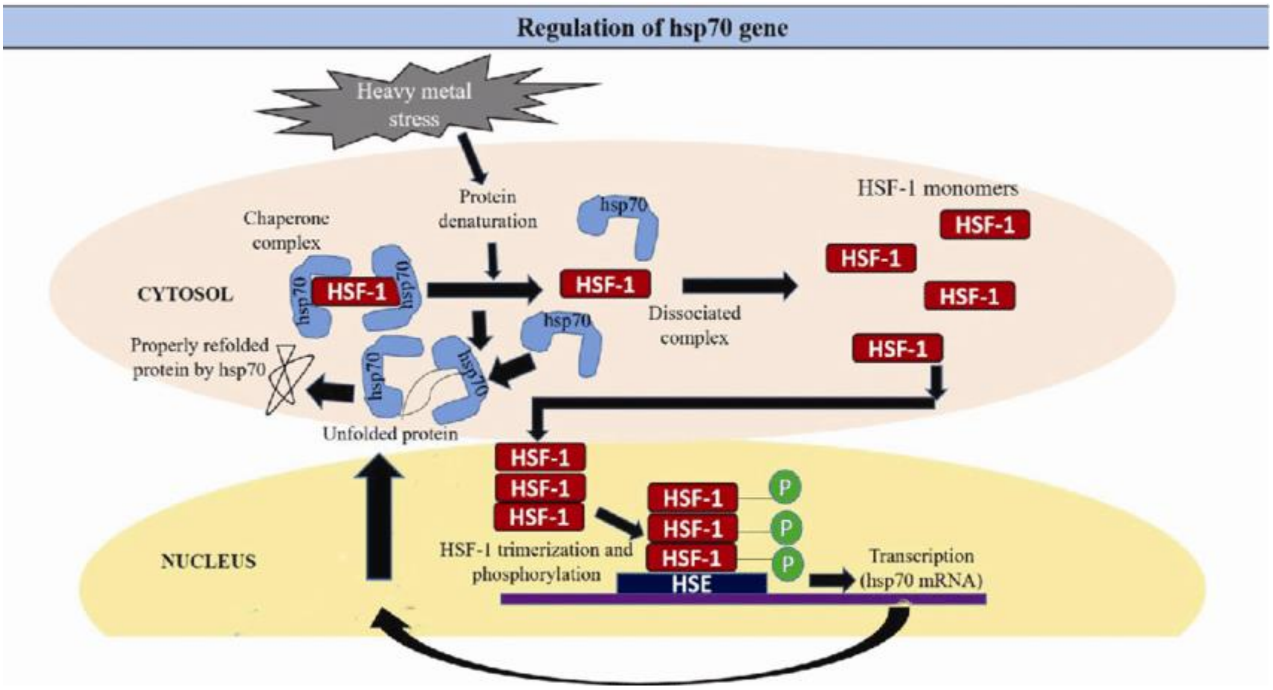

Figura 3. Desenho esquemático da indução de genes HSP70 sob contaminação por metais pesados (Somasundaram et al., 2018).

1.4 Citocromo P450

O citocromo P450 (CYP) representa uma família de enzimas responsável pela biotransformação de compostos orgânicos. A sub-família CYP1A tem alta afinidade por hidrocarbonetos policíclicos aromáticos que dependem basicamente do receptor AHR (Aryl Hydrocarbon Receptor). A indução inicia-se pela ligação do xenobiótico específico ao AHR, que atrai uma proteína de choque térmico 90 (HSP90), e se movimenta do citoplasma ao núcleo da célula (Kawajiri & Fujii-Kuriyama, 2007). No núcleo, a HSP90 se dissocia do complexo receptor-indutor. Após a liberação da HSP90, o complexo receptor-indutor liga-se a outra proteína, o translocador de receptor de hidrocarbonetos aromáticos (ARNT), que também se localiza no núcleo da célula. No núcleo, o complexo ARNT-AHR liga-se a uma região específica do DNA, o elemento de resposta à xenobiótico (XRE). Fatores transcricionais são utilizados para acessar a região promotora do gene CYP1A, e o RNA mensageiro é então sintetizado. Essa ativação gênica provoca um aumento na expressão e na atividade da enzima CYP1A, levando então a biotransformação, que resulta na detoxificação/bioativação dos contaminantes orgânicos (Figura 4) (Safe & Krishnan, 1995; Kawajiri & Fujii-Kuriyama, 2007).

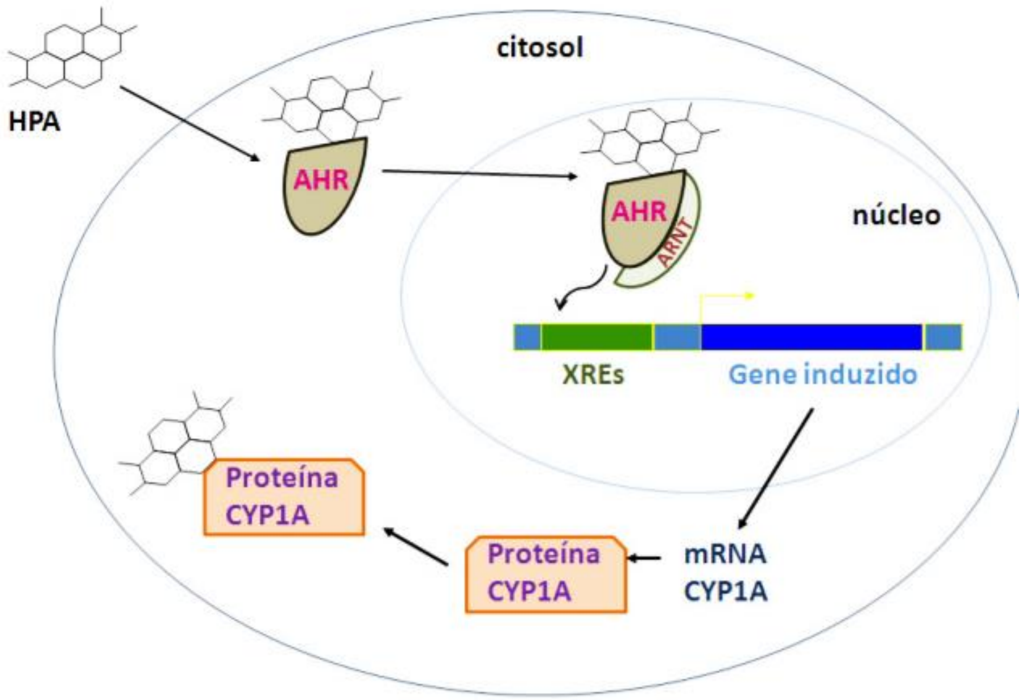

Figura 4. Desenho esquemático da indução do gene CYP1A na presença de um hidrocarboneto policíclico aromático (HPA) (Kawajiri & Fujii-Kuriyama, 2007).

1.5 Espécie em estudo

O *Salminus franciscanus* (Lima & Britski, 2007), anteriormente classificado como *Salminus brasiliensis* (Cuvier, 1816), é conhecido popularmente como dourado (Figura 5). É um peixe da família Bryconidae, sub-família Salmininae, endêmico da bacia do rio São Francisco, Minas Gerais. O dourado tem grande importância comercial e ecológica, podendo atingir mais de 1,0 metro de comprimento total, ultrapassando 25kg de peso corporal, com hábito alimentar essencialmente piscívoro, ocupando o topo da cadeia alimentar nos rios, o que favorece a bioacumulação de metais pesados. Em relação à biologia reprodutiva do dourado Freitas et al. (2013) observou que o peixe reproduz no alto e médio dos rios, no período de outubro a fevereiro, onde o fotoperíodo é maior, a água está mais quente e com maior taxa de oxigênio dissolvido, favorecendo a desova do tipo total, com ovos não-adesivos e alta fecundidade. Atualmente constitui um dos principais alvos da pesca comercial e esportiva (Petrere 1989; Sato & Godinho, 2003) e devido à crescente degradação ambiental e barramentos nos rios da bacia do rio São Francisco por usinas hidrelétricas e termelétricas, suas populações têm sido ameaçadas (Sato & Sampaio, 2005, Freitas et al., 2013).

Figura 5 – Exemplar de dourado, *Salminus franciscanus* (comprimento total - 55,0cm).

1.6 Área de estudo

O rio Paraopeba é considerado um dos principais afluentes do rio São Francisco, MG, sua nascente está localizada no município de Cristiano Otoni, MG, e sua foz na represa de Três Marias, município de Felixlândia, MG. Possui uma área de 13.643 km^2 e extensão aproximada de 510 km (IGAM, 2012[a]). É classificado como uma área prioritária para a conservação, devido à alta diversidade de espécies endêmicas de peixes, reprodução de espécies de piracema e/ou por ser ambiente único no Estado (Costa et al., 1998), porém atualmente vêm sendo ameaçado pela contaminação por resíduos de galvanoplastia das indústrias de aço, resíduos da agricultura, curtumes, esgoto (doméstico e industrial) e rejeitos minerários, incluindo contaminantes como metais pesados, que excedem os níveis de segurança para a saúde humana, além da presença em seu curso, de uma usina termoelétrica e uma hidrelétrica.

Entretanto o rio Abaeté, com sua nascente localizada na Serra da Canastra, município de São Gotardo, MG e sua foz no rio São Francisco, MG, em um local denominado como Pontal do Abaeté, apresenta melhor qualidade ambiental, sendo um ambiente mais preservado de atividades antrópicas, apresentando pouca contaminação ambiental (IGAM, 2012[b]). Também apresenta parâmetros físico-químicos adequados para o sucesso reprodutivo dos peixes, criando um ambiente propício e único para a manutenção da ictiofauna na bacia do rio São Francisco, MG (Arantes et al., 2010; Weber, et al., 2013; Nunes et al., 2015). Neste contexto, fica evidente a necessidade de estudos voltados ao diagnóstico da atual situação da ictiofauna na bacia do rio São Francisco, MG, e de sua sanidade. Devendo estes estudos, fornecer informações sobre como os contaminantes estão presentes, e suas consequências, para com o ambiente natural, possibilitando constituir ações prioritárias de instituições de pesquisa e fiscalização ambiental.

2. JUSTIFICATIVA

O ambiente aquático está cada vez mais contaminado por resíduos industriais e domésticos, incluindo metais pesados, que posteriormente tendem a ser bioacumulados nos peixes e em toda a fauna aquática. Essa bioacumulação promove diversas alterações biológicas negativas no organismo vivo e os efeitos, ainda pouco descritos na literatura, precisam ser melhor investigados. A carência de estudos dessa natureza no Brasil, demonstra a necessidade de um maior número de pesquisas científicas nessa área de toxicologia ambiental e seus efeitos.

Na bacia do rio São Francisco, MG, estudos recentes vêm sendo realizados registrando altas concentrações de metais pesados bioacumulativos em peixes de interesse comercial, tornando os peixes impróprios para consumo humano, além do desenvolvimento de diversas alterações histopatológicas em órgãos alvo, e alterações reprodutivas. Estudos toxicológicos em ambientes contaminados, como aqui realizado, nos proporcionam utilizar o peixe como modelo experimental para se avaliar efeitos dos poluentes aquáticos sobre o organismo dos peixes, avaliando em conjunto a qualidade ambiental e fatores que afetam a saúde humana, como o consumo da carne contaminada por metais pesados.

3. OBJETIVOS

3.1 Objetivo geral

Avaliar a contaminação por metais pesados sobre alterações histopatológicas e expressão de proteínas biomarcadoras de impacto ambiental, em órgãos-alvo como fígado, baço e músculo, do dourado, *Salminus franciscanus,* coletados em dois rios da bacia do rio São Francisco, MG: Paraopeba e Abaeté.

3.2 Objetivos específicos

- Calcular os Ìndices: Gonodassomático (IGS), Hepatossomático (IHS) e Fator de Condição de Fulton (K), em machos e fêmeas;
- Comparar os valores de IGS, IHS e K entre os trechos de amostragem A e B do rio Paraopeba, com o rio Abaeté;
- Dosar as concentrações de metais pesados (Chumbo, Cobre, Cromo, Cádmio, Ferro e Zinco) em fragmentos de fígado e músculo dos peixes coletados nos diferentes trechos de amostragem;
- Correlacionar as concentrações de metais pesados obtidas com os valores seguros ao consumo humano (OMS/ANVISA);
- Detectar e quantificar as principais alterações histopatológicas no fígado e baço dos peixes;
- Avaliar as alterações na expressão de Metalotioneína (MT), Proteínas de Choque Térmico-70 (HSP70) e Citocromo P450-1A (CYP1A) no fígado dos peixes, via Imunohistoquímica Peroxidase;
- Imunolocalizar MT, HSP70 e CYP1A no fígado, através de técnicas Imunohistoquímicas Peroxidase;
- Comparar alterações histopatológicas e expressão de biomarcadores de qualidade ambiental entre os trechos de amostragem do presente estudo.

4. ARTIGO SUBMETIDO

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Assessment of environmental heavy metal pollution and potential human health risk in a highly consumed fish, Salminus franciscanus, using an immunohistochemical and histopathological approach

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ASSESSMENT OF ENVIRONMENTAL HEAVY METAL POLLUTION AND POTENTIAL HUMAN HEALTH RISK IN A HIGHLY CONSUMED FISH, *SALMINUS FRANCISCANUS*, USING AN IMMUNOHISTOCHEMICAL AND HISTOPATHOLOGICAL APPROACH

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ABSTRACT

Due to production of industrial, rural and domestic waste, heavy metals such as cadmium (Cd), chrome (Cr), lead (Pb), zinc (Zn), copper (Cu) and iron (Fe) constantly reach aquatic environments. The present study uses histological and immunohistochemical analyses to evaluate the contamination status of *Salminus franciscanus,* a large fish of great economic importance. Levels of Cd, Cr, Pb, Zn, Cu and Fe were evaluated for liver and muscle tissue of 68 fish, from two tributaries of the upper São Francisco River Basin, Brazil: Abaeté and Paraopeba. In addition, histopathological alterations and expression of three biomarkers — metallothionein (MT), heat shock protein-70 (HSP70) and cytochrome P450-1A (CYP1A) — were assessed. Fish from the Paraopeba River were found to be inappropriate for human consumption because they exceed safe limits established by the World Health Organization (WHO). Histopathological alterations in the liver and spleen were significantly more frequently in fish from the Paraopeba River ($P<0.05$). Significant differences were also observed in the levels of expression of MT, HSP70 and CYP1A proteins, regarding the heavy metal contamination levels. Fish from Abaeté River, on the other hand, had higher values for IGS and low levels of metal contamination in liver and muscle, and thus can be considered healthy for consumption and population sustainability. We emphasize the socio-environmental importance of *S. franciscanus* as an excellent environmental bioindicator.

Keywords: Bioaccumulation; Histopathology; Metallic ions; Teleosts; Trace elements; Water contamination.

INTRODUCTION

Toxicological studies have recently reported increases in the contamination of aquatic environments by heavy metals, mainly from activities such as mining, agriculture and industry (Squadrone et al. 2013; Santolin et al. 2015; Zhong et al. 2018; Jiang et al., 2019). Heavy metals do not degrade and thus tend to accumulate in organs and tissues of fish and other aquatic organisms (D'Costa et al. 2017; Lunardelli et al. 2018). At high concentrations these metals can also disrupt ion homeostasis, induce DNA damage, and cause histopathological alterations and disorders in different organs (Vieira et al. 2011; Nai et al. 2015). This accumulation of toxic compounds in organisms causes pathologies in target organs, such as steatosis in the liver, fibrosis in the spleen, lesions in the cranial portion of the kidneys and necrosis and cancer in several organs (Vieira et al. 2011; Thévenod and Lee, 2015). Chronic exposure to heavy metals can also increase levels of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide radical (O_2^-) and hydroxyl radical (OH^-), thus triggering oxidative stress and cell death (Ercal et al. 2001; Ratn et al. 2018; Ibor et al. 2019).

Liver and spleen are organs involved in intense metabolism and detoxification. As such, they can accumulate certain pollutants, especially heavy metals, and thus are suitable targets for toxicological studies (Alm-Eldeen et al. 2018). The expression of certain liver proteins, especially metallothioneins (MTs) can be altered by exposure to metals. These proteins are characterized by having low molecular weight and being cysteine-rich. Moreover, they are important for homeostasis of essential metals and detoxification of toxic metals by protecting against oxidative damage and toxicity resulting from excessive exposure to heavy metals (Chan, 1995; Ruttkay-Nedecky et al. 2013, Sevcikova et al. 2013; Le et al. 2016). Due to their specificity and ability to bind to metal ions, MTs are considered suitable biomarkers for monitoring aquatic environments contaminated by heavy metals (Rajeshkumar et al. 2013).

Several biomarkers are widely used in toxicological and biomonitoring studies to assess environmental stress in aquatic organisms, including heat shock proteins (HSPs) and some cytochrome P450 family proteins, such as CYP1A. Heat shock proteins are molecular chaperones that act in the formation and restructuring of proteins in the body (Feder and Hofmann, 1999; Parcellier et al. 2003). Among the proteins of this family, HSP70 is a highly conserved within vertebrates and the first to be induced in response to several stressors (Mukhopadhyay et al. 2003). Heavy metals can accumulate in cells and interact with the thiol group of proteins, generating an abnormal or denatured protein, consequently up-regulating the expression of HSP70 (Somasundaram et al. 2018). CYP1A is a member of the xenobiotic family of metabolic enzymes, acts on the detoxification of contaminants in organs of intense metabolism (i.e., liver), and has high affinity for polycyclic aromatic hydrocarbons that basically depend on the aryl hydrocarbon receptor-AHR (Safe and Krishnan 1995; Kawajiri and Fujii-Kuriyama, 2007).

The Abaeté and Paraopeba Rivers are important tributaries for the maintenance of native fish stocks in the São Francisco River, an important river basin in South-America (Domingos et al. 2012). The Abaeté River has low levels of environmental contamination and adequate water quality parameters for the successful reproduction of migratory fish, and thus contributes to maintaining ichthyofauna (Arantes et al. 2010; Weber et al. 2013; Nunes et al. 2015; Paschoalini et al. 2019). However, high levels of domestic and agricultural sewage, and effluents from mining operations and tanneries have been reported for the Paraopeba River, which negatively affect fish species of high commercial value (IGAM 2012A; Arantes et al. 2016; Savassi et al. 2016; Paschoalini et al. 2019).

Salminus franciscanus (Lima and Britski, 2007), popularly known as dourado, is a large piscivorous and highly consumed fish. The species breeds in the rainy season, from October to February, exhibiting total spawning, non-adhesive eggs and high fecundity (Freitas et al. 2013). The goal of the present study was to use histopathological analyses and protein expression of biomarkers of environmental impact to evaluate heavy metal contamination of *S. franciscanus* from two tributaries of the São Francisco River Basin in southeastern Brazil.

MATERIALS AND METHODS

Sampling

Specimens of *S. franciscanus* were sampled from the Abaeté and Paraopeba Rivers during the breeding seasons (October to February) of 2010, 2011 and 2012. Samples from the Paraopeba River were taken at two distinct sites: Site A - downstream of the Igarapé thermoelectric plant (19°57'50.24"S, 44°16'52.42"W); and Site B - downstream of the Retiro Baixo power plant (18°52'28.64"S, 44°46'50.27"W). Samples from the Abaeté River were taken from a single site, considered reference site in present study (18°05'07.8"S, 45°17'00.1"W). All procedures performed during fish collection followed the ethical principles established by the Conselho Nacional de Controle de Experimentação Animal (CONCEA). The study was developed with approval by the Animal Use Ethics Committee of the Pontifical Catholic University of Minas Gerais (CEUA-PUC Minas-protocol 021/2015).

A total of 68 adult specimens were used in this study: 36 fish from the Paraopeba River (18 from Site A and 18 from Site B), and 32 from the Abaeté River. All fish were euthanized with a lethal dose of Eugenol (200mg /l). Total length (TL), body weight (BW), weight of gonads (GW) and liver weight (LW) were measured for each specimen.

To standardize the analyses, and due to the sexual dimorphism exhibited by this species, only females with total length (TL) between 60 and 87 cm and body weight (BW) between 2250 and 8400g, and males with TL between 58 and 84 cm and BW between 2050 and 7980g, were used.

Water quality

Water quality data for the sampled sites were obtained from quarterly reports of Instituto Mineiro de Gestão das Águas (IGAM), which has monitoring points coinciding with the sites sampled in the present study (Tables 1 and 2). The raw data obtained from IGAM are available for public consultation and were used in the present study as water quality parameters for the two rivers (IGAM, 2012B). Legislation established by the Canadian Environment Council (CCME, 2011) was adopted as a reference for permissible levels of heavy metals in water because it is among the most rigorous and current of such legislations.

Biological indices

Gonadosomatic (GSI = GW / BW x 100) and hepatosomatic (HSI = LW / BW x 100) indices were calculated to analyze changes in the volume of gonads and liver related to body weight. Only males and females in the advanced maturation stage were used in this study. The Fulton condition factor (K = BW / TL3 x 100) was also calculated to compare the health status of males and females between the rivers.

Histology and immunohistochemistry

Liver and spleen samples from 21 specimens, seven per sampled river Site, of *S. franciscanus* were used for immunohistochemical reactions. The samples were fixed in

Bouin solution for 24 h at room temperature, embedded in paraffin, submitted microtomy at 5-µm thickness, and stained with hematoxylin-eosin and Gomori trichrome.

The following primary antibodies were used: anti-MT (Abcam 1:50); anti-HSP70 (Sigma 1: 100); and anti-CYP1A (Biosense 1:50). The sections were hydrated and washed for 60 min under running water. Blocking of endogenous peroxidase was performed using 3% hydrogen peroxide (H2O2) diluted in TBS.

Antigenic recovery occurred in sodium citrate buffer (pH 6.0) for 30 min in a water bath at 60 ° C. Blocking buffer (2% BSA + 0.05% Triton X-100 + 0.01% Tween 20) was used for permeabilization and blocking of non-specific reactions. Primary antibodies were applied using their respective dilutions and remained in the humid chamber for 15 h (overnight) at 4 ° C. After washing with TBS, Dako EnVision + Dual Link System HRP, (100µl per slide) was applied. The reaction was revealed using 3'-diaminobenzidine (DAB) and the sections were counterstained with hematoxylin for 30 s. To ensure success in the procedure, the primary antibodies were not used in the negative controls.

Histopathological assessment

For quantification of histopathological alterations in liver, 10 fish per river site were chosen at random. Six random images of each specimen were analyzed at 200X magnification, for a total of 60 images per river site. Using the grid function with 494 points of intersection of Image J software, the following histopathological changes were counted: sinusoidal congestion, sinusoidal dilatation, pigmented macrophages and vacuolization of hepatocytes. In addition, blood vessels and parenchyma were also quantified.

For quantification of fibrosis in spleen, 10 fish per river site were chosen at random. Six random images of each specimen were analyzed at 100X magnification, for a total of 60 images per river site. The images were analyzed using the IHC Toolbox function of Image J software. The Image J software dropper tool was used to remove the background and isolate the region to be quantified (connective tissue stained by light green). The image was then transformed into a binary image, and the program read the specific wavelength for the chosen pigment. The results of this analysis were expressed as percentage of marked area.

Immunohistochemical assessment

Immunoperoxidase reactions for MT, HSP70 and CYP1A were analyzed using the IHC Toolbox, function of Image J software (Gupta et al. 2007). The software provides a dropper tool function that permits manual identification of the pigment to be analyzed (regions stained by dark brown from positive reactions to DAB). The software function was used to remove the background and isolate the region of interest. Images were then transformed into binary images for quantification. The results are expressed as percentage of marked area out of the total area of the image.

Heavy metal analysis

Concentrations of copper (Cu), lead (Pb) cadmium (Cd), zinc (Zn), chromium (Cr) and iron (Fe) were determined by submitting samples of liver and muscle to acid decomposition by microwave (US-EPA 351-A) StartD (Milestone) with controlled heating. Samples of approximately 1.5 g, in triplicate, were weighed and placed in PTFE-TFM pumps with 20 mL of nitric acid (65% HNO3) and digested for 25 min at 220°C. After acid digestion, the samples and blank solutions were analyzed in an atomic absorption spectrophotometer with flame and graphite furnace (model iCE 3500 - Thermo Fisher Scientific). To ensure analytical quality of the results and validation of the method, standard fish protein DORM 2 (fish protein with certified reference material for trace metals) was analyzed together with the study material.

Statistical analyses

Statistical analyses were performed using GraphPad Prism software version 5.0 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com). The results were expressed as mean ± standard error (S.E.M.) and considered significant in the 95% confidence interval ($p < 0.05$). Since both biological data and metal concentrations were not normally distributed, non-parametric Kruskal-Wallis tests were used followed by Dunn and Mann-Whitney post-tests (Wilcoxon test).

RESULTS

The physico-chemical water quality parameters of the Paraopeba River were similar to those of the Abaeté River with the exception of conductivity, which was significantly higher in the Paraopeba River (Table 1). In addition, metals, including as Pb, Zn, Cr and Fe, were at higher concentrations than the limits allowed by the Canadian

Environmental Council (CCME 2011). In contrast, none of the analyzed heavy metals exceeded the established safe limits in the Abaeté River, (Table 2).

Regarding biological indices, mean GSI values were higher for males and females of the Abaeté River than for fish from the Paraopeba River ($p < 0.05$). Likewise, HSI was significantly higher for fish of the Paraopeba River than for fish of the Abaeté River ($p < 0.05$). The Fulton K condition factor was closer to 1.00 for fish of the Abaeté River, differing significantly from the values for Paraopeba fish ($p < 0.05$). No significant differences were observed for HIS and K between sites A and B of the Paraopeba River (Table 3).

Histopathological analyses revealed morphological alterations in hepatic and splenic tissues, with higher incidences for fish caught in the two sites of the Paraopeba River. Alterations to the liver included areas of congestion and dilation of sinusoidal capillaries, large numbers of pigmented macrophages associated with blood vessels, infiltration of inflammatory cells in vascularized regions, and hepatocytes with cytoplasmic vacuolization, displacement and nuclear flattening (Fig. 1).

Quantification of histopathologies revealed that congestion of the sinusoids varied statistically among the three sampled river sites, with there being a greater amount for fish from site A of the Paraopeba River, while the highest percentage for dilation of sinusoids was observed in fish from PBR B. Pigmented macrophages were not detected in samples from the Abaeté River, but a significant percentage of these cells was observed in the two sites of the Paraopeba River. Cytoplasmic vacuolization in hepatocytes also differed significantly between the Abaeté and Paraopeba Rivers, higher in Paraopeba sites. The occurrence of blood vessels and liver parenchyma were also quantified and had higher percentage in the Abaeté River (Fig. 2).

Histological analysis of spleen revealed extensive areas of fibrosis in the trabeculae of the organ, indicating an increase in interstitial stroma and a decrease in the parenchyma in fish from the two sites of the Paraopeba River. In addition, the presence of pigmented macrophages associated with blood vessels and increased connective tissue was observed in fish from Paraopeba sites (Fig. 3). There were more areas of fibrosis in the spleen of fish from the Paraopeba River (Fig. 4), and more in PBR A than PBR B.

Molecular analysis of biomarkers also revealed significant differences between the sampled sites of the present study. The expression of MT occurred in granules dispersed in the cytoplasm of hepatocytes and in some cases in close association with blood vessels and near areas of vacuolation in hepatocytes (Fig. 5). The MT levels were higher in site A than in site B of the Paraopeba River, with significant differences in relation to those of Abaeté River (Fig. 6). The markers HSP70 and CYP1A presented different patterns of expression than MT, with more scattered markers in the cytoplasm (Fig. 5). Quantification of HSP70 revealed no significant differences between sites of the Paraopeba River, but significantly higher levels in the Abaeté River ($p < 0.05$) (Fig. 6). As with MT and HSP70, CYP1A had higher levels in the Paraopeba River, with the levels being higher in PBR A presented higher values than PBR B ($P < 0.05$) (Fig. 6). Negative immunoperoxidase controls did not show any protein expression, ensuring the reliability of the technique (Fig. 5).

The analysis of heavy metals in fragments of liver and muscle of *S. franciscanus* revealed values that exceeded the safe limits for human consumption established by the World Health Organization (WHO 2002) and the National Health Surveillance Agency (ANVISA 2013) (Table 4). For the Abaeté River, only Cr, in both liver and muscle, was detected at levels above the legal limit. In the Paraopeba River, on the other hand, heavy metals including Cu, Pb, Cd, Cr and Fe had values that exceeded the safe limits allowed by the two agencies. The concentrations of all metals in the liver of fish from the Paraopeba River were at levels above safe limits, and were higher than the levels in muscular tissue, which were still, with the exception of zinc, above the safe limits (Table 4).

DISCUSSION

A major contemporary concern worldwide is the correct disposal of pollutants produced on a large scale (Jaishankar et al. 2014; Li and Xie 2016). Untreated effluents released directly into water bodies cause damage to several organisms, especially in fish (Wilhelm et al. 2017). This scenario was apparent in the Paraopeba River, where the results of the present study corroborated other studies conducted in the same River, such as Arantes et al. (2016), Savassi et al. (2016) and Paschoalini et al. (2019), who demonstrated morphophysiological alterations in different species of fish due to heavy metal contamination. However, the Abaeté River, also analyzed in the present work, presents better environmental conditions, with low levels of pollutants (IGAM 2012A; Procópio et al. 2014; Paschoalini et al. 2019). We emphasizing that in the present study innovations on the expression of biomarkers of environmental pollution and other aspects related to histopathology were obtained.

Physico-chemical parameters and biometric data did not differ significantly among the river sites sampled in the present study. Standardization of these parameters in comparative toxicological analyses results in more reliable data, since the metabolic capacity and the appearance of histopathologies in fish may be related to water temperature, pH, dissolved oxygen, size and body weight (Canli and Atli 2003; Polat et al. 2015; Sassi et al. 2010). Among the biological indices evaluated, GSI had higher values in the Abaeté River, which is in agreement with recently published studies that showed this river to possess favorable conditions for successful reproductive of different species of teleosts (Arantes et al. 2010; Weber et al. 2013; Nunes et al. 2015; Paschoalini et al. 2019). On the other hand, HSI, unlike GSI, was found to have higher values in environments contaminated by metals, namely sites A and B of the Paraopeba River. This increased HSI may be related to increased organ detoxification activity (Pyle et al. 2005; Querol et al. 2002). The Fulton condition factor tends to be lower in higher quality environments (Fang et al. 2009; Weber et al. 2017), as observed in the present study.

Histopathological analyses are widely used to detect physiological changes and to diagnose the health of fish exposed to pollutants in a chronic or acute manner (Camargo and Martinez 2007; Costa et al. 2011; Kaur et al. 2018). Histopathological findings such as those for the liver of fish from site A and B of the Paraopeba River have also been reported for other freshwater species from other environments impacted by heavy metals (Jaishankar et al. 2014; Savassi et al. 2016; Ratn et al. 2018). Sinusoid congestion, dilatation, and infiltration of inflammatory cells in the liver of fish contaminated by heavy metals occurred at significantly higher levels, indicating the negative influence of these elements on the organism as showed by Poleksic et al. (2010). Likewise, high levels of fibrosis like those recorded for the spleens of fish from Paraopeba River have been reported in other environments, where fish are exposed to heavy metals in recent studies, significantly associating heavy metal contamination on the incidence of fibrosis in fish spleen (Handy et al. 2002; Salim et al. 2015).

The results of the histopathological analyses and the concentrations of heavy metals in liver and muscle coincide with the results obtained by the immunohistochemical tests, with MT, HSP70 and CYP1A levels being higher in fish from the Paraopeba River. These results demonstrate that histopathological analyses associated with molecular evaluation methods are necessary for accurately diagnosing the class of pollutants acting on organisms, as reported by Rajeshkumar et al. (2017). Studies of the effects of heavy metal contamination on histopathological alterations and protein expression in organism, as performed here, reflect the actual condition of the environmental contamination status and can be applied worldwide, contributing to a better understanding of this environmental problem.

Hepatic expression of MT coincided with the levels of heavy metals detected in the fish collected in the two studied rivers. Heavy metal contamination in Paraopeba River was higher in site A than site B, corroborating with MT expression, which was higher in site A, followed by site B and Abaeté River, respectively. The significantly higher levels of MT observed in sites A and B of the Paraopeba River indicate greater exposure of *S. franciscanus* to metallic ions and confirm the effectiveness of this biomarker in the detection of metallic pollutants in aquatic environments (Le et al. 2016; Mani et al. 2014).

The results for the environmental stress biomarkers reinforce the idea that heavy metals, detected at high concentrations in Paraopeba River, may promote the formation of abnormal proteins, since significant increases in the expression of HSP70 in PBR were detected. Similar results were observed by Rajeshkumar et al. (2013) and Somasundaram et al. (2018), who found heavy metals to be responsible for triggering the activation cascade of this protein. In contrast to HSP70, CYP1A was expressed in a manner similar to MT, with significant differences between the sites of the Paraopeba River. Despite the lack of studies correlating heavy metals and CYP1A in freshwater fish, our results indicate an increase in the expression of this biomarker in environments contaminated by metals. These results corroborate with Anwar-Mohamed et al. (2009), who showed that heavy metals can regulate the expression of CYP1A in different aryl-hydrocarbon receptor (AHR) signaling pathways in a metal-dependent manner.

The analysis of heavy metals in hepatic and muscular tissues revealed higher levels of contamination of the liver than of the muscle, which is expected since organs of intense metabolism tend to exhibit higher levels of contamination (Eneji et al. 2011; Taweel et al. 2013). In addition, levels of heavy metals that exceed the limits allowed for food by the World Health Organization (WHO 2002), have been previously recorded in the muscle tissue of fish from the Paraopeba River (Arantes et al. 2016; Paschoalini et al. 2019), which represents a potential danger to the health of any population that consumes *S. franciscanus* extensively.

The Paraopeba River was classified as a priority conservation area due to its high diversity of native fish species (Drummond et al. 2005). Nonetheless, the data of the present study, along with those of other studies, reveal that the toxic condition of this river negatively influences the biology of different species of fish (Arantes et al. 2016;

Savassi et al. 2016; Paschoalini et al. 2019). Additionally, on January 24, of 2019, a mining tailings dam on the Paraopeba River broke, releasing a massive load of heavy metals into the river, which will certainly contribute to increasing the toxic condition of the river with negative impacts for the conservation and maintenance of its ichthyofauna. In summary, the results of the present study are essential for the continuity of research in aquatic ecosystems contaminated by heavy metals, highlighting the importance of studies about the use of piscivorous fish as an experimental model to evaluate the levels of heavy metal contamination and its effects on the organism, since the natural environment is increasingly contaminated by these inorganic pollutants.

CONCLUSIONS

The present study indicates that the bioaccumulation of heavy metals may be related to the higher occurrence of histopathological alterations in the liver and spleen of fish. These changes, associated with significant increase expression of MT, HSP70 and CYP1A in fish liver, suggest that these biomarkers can be important in the assessment of environmental quality in ecosystems contaminated by heavy metals. The study provides important and current information of the action of heavy metals on the organism, especially in the use of environmental impact biomarkers associated with histological tools. In summary, the data obtained in the present study show that heavy metals may negatively influence the biology of *Salminus franciscanus* and human health through the consumption of contaminated fish.

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REFERENCES

Alm-Eldeen AA, Donia T, Alzahaby S. 2018. Comparative study on the toxic effects of some heavy metals on the Nile Tilapia, *Oreochromis niloticus*, in the Middle Delta, Egypt. Environ Sci Pollut 25(15), 14636–14646. doi:10.1007/s11356-018-1677-z

ANVISA - Agência Nacional de Vigilância Sanitária. 2013. RDC n° 42 de 29 de Agosto de 2013 - Dispõe sobre o Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimentos. DOU N168 33p

Anwar-Mohamed A, Elbekai RH, El-Kadi AO. 2009. Regulation of CYP1A1 by heavy metals and consequences for drug metabolism. Exp Opi on Drug Meta Toxi, 5(5), 501–521. doi:10.1517/17425250902918302

Arantes FP, Santos HB, Rizzo E, Sato Y, Bazzoli N. 2010. Collapse of the reproductive process of two migratory fish (*Prochilodus argenteus* and *Prochilodus costatus*) in the Três Marias Reservoir, São Francisco River, Brazil. J of App Ichthyol 27, 847 - 853. doi: 10.1111/j.1439-0426.2010.01583

Arantes FP, Savassi LA, Santos HB, Gomes MVT, Bazzoli N. 2016. Bioaccumulation of mercury, cadmium, zinc, chromium, and lead in muscle, liver, and spleen tissues of a large commercially valuable catfish species from Brazil. An Acad Bras Cienc 88, 137–147. doi:10.1590/0001-3765201620140434

Camargo MMP and Martinez CBR. 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotrop Ichthyo. 5, 327–336. doi:10.1590/S1679-62252007000300013

Canli M and Atli G. 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ Pollut 121, 129–136. doi:10.1016/S0269-7491(02)00194-X

CCME - Canadian Council of Environment. 2011. Canadian water quality guidelines for the protection of aquatic file: CCME Wanter Quality Index 1.0, User´s Manual. Canadian environmental quality guidelines

Chan KM. 1995. Metallothionein: potencial biomarker for monitoring heavy metal pollution in fish around Hong Kong. Mar Pollut Bull v. 31, p. 411-415

Costa PM, Caeiro S, Lobo J, Martins M, Ferreira AM, Caetano M, Vale C, DelValls TA, Costa MH. 2011. Estuarine ecological risk based on hepatic histopathological indices from laboratory and in situ tested fish. Mar Pollut Bull 62, 55–65. doi:10.1016/j.marpolbul.2010.09.009

D'Costa A, Shyama SK, Kumar P. 2017. Bioaccumulation of trace metals and total petroleum and genotoxicity responses in an edible fish population as indicators of marine pollution. Ecotox Environ Safe 142. 22-28. 10.1016/j.ecoenv.2017.03.049

Domingos FFT, Thomé RG, Arantes FP, Castro ACS, Sato Y, Bazzoli N, Rizzo E. 2012. Assessment of spermatogenesis and plasma sex steroids in a seasonal breeding teleost: a comparative study in an area of influence of a tributary, downstream from a hydroelectric power dam, Brazil. Fish Physiol Biochem 38(6), 1709–1719. doi:10.1007/s10695-012-9668-3

Drummond GM, Martins CS, Machado ABM, Sebaio FA, Antonini Y. 2005. Biodiversidade em Minas Gerais: um atlas para sua conservação, Biodiversidade em Minas Gerais: um atlas para sua conservação

Eneji IS, Sha'Ato R, Annune PA. 2011. Bioaccumulation of Heavy Metals in Fish (*Tilapia zilli* and *Clarias gariepinus*) Organs from River Benue, North-Central Nigeria. Pakistan J Analy Environ Chem 12, 25–31

Ercal NBSP, Gurer-Orhan HBSP, Aykin-Burns NBSP. 2001. Toxic Metals and Oxidative Stress Part I: Mechanisms Involved in Me-tal induced Oxidative Damage. Current Topic Med Chem 1(6), 529–539.doi:10.2174/1568026013394831

Fang JKH, Au DWT, Wu RSS, Chan AKY, Mok HOL, Shin PKS. 2009. The use of physiological indices in rabbitfish *Siganus oramin* for monitoring of coastal pollution. Mar Pollut Bull 58(8), 1229–1235. doi:10.1016/j.marpolbul.2009.05.013

Feder ME and Hofmann GE. 1999. Heat-shock proteins, molecular chaperones and the stress response: evolutionary and ecological physiology. Annual Review Physiol v. 61, p. 243-282

Freitas LJA, Prado PS, Arantes FP, Santiago KB, Sato Y, Bazzoli N, Rizzo E. 2013. Reproductive biology of the characid dourado *Salminus franciscanus* from the São Francisco River, Brasil. Ani Reprod Sci v.139, p. 145–154

Gupta GP, Nguyen DX, Chiang AC, Bos PD, Kim JY, Nadal C, Gomis RR, Manova-Todorova K, Massague J. 2007. Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. Nature 446, 765–770. doi:10.1038/nature05760

Handy RD, Runnalls T, Russell PM. 2002. Histopathologic biomarkers in three spined sticlebacks, *Gasterosteus aculeatus* from several rivers in southern England that meet the Freshwater Fisheries Directive. Ecotoxicology 11, 467-479

Ibor OR, Adeogun AO, Regoli F, Arukwe A. 2019. Xenobiotic biotransformation, oxidative stress and obesogenic molecular biomarker responses in Tilapia guineensis from Eleyele Lake, Nigeria. Ecotox Environ Safe 169, 255–265. doi:10.1016/j.ecoenv.2018.11.021

IGAM[A] - Instituto Mineiro de Gestão das Águas. 2012. Estudos das Metas de Qualidade da Bacia Hidrográfica do Rio Paraopeba. Avaliable in: http://comites.igam.mg.gov.br/comites-estaduais/bacia-do-rio-sao-francisco/sf3-cbh-do-rio-paraopeba/1104-conheca-a-bacia. Acess in march / 2015

IGAM[B] - Instituto Mineiro de Gestão das Águas. 2012. Monitoramento da qualidade das águas superficiais no estado de Minas Gerais, Brasil. Quarterly report avaliable in: http://www.igam.mg.gov.br/images/ stories/ qualidade/2013/relatorio-trimestral-am-3o-trim-2012.pdf

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 7(2), 60–72. doi:10.2478/intox-2014-0009

Jiang D, Wang Y, Zhou S, Long Z, Liao Q, Yang J, Fan J. 2019. Multivariate analyses and human health assessments of heavy metals for the surface water quality in the Xiangjiang River Basin, China. Environ Toxicol Chem. 00(00) 1-13. doi:10.1002/etc.4461

Kaur S, Khera KS, Kondal KJ. 2018. Heavy metal induced histopathological alterations in liver, muscle and kidney of freshwater cyprinid, *Labeo rohita* (Hamilton). J Entomol Zool Stud 6(2): 2137-2144

Kawajiri K and Fujii-Kuriyama Y. 2007. Cytochrome P450 gene regulation and physiological functions mediated by the aryl hydrocarbon receptor. Archiv Biochem Biophy 464. 207-12. 10.1016/j.abb.2007.03.038

Le TTY, Zimmermann S, Sures B. 2016. How does the metallothionein induction in bivalves meet the criteria for biomarkers of metal exposure? Environ Pollut 212, 257–268. doi:10.1016/j.envpol.2016.01.070

Li J and Xie X. 2016. Heavy Metal Concentrations in Fish Species from Three Gorges Reservoir, China, After Impoundment. Bull Environ Cont Toxicol 96(5), 616–621. doi:10.1007/s00128-016-1772-0

Lima FCT and Britski HA. 2007. *Salminus franciscanus*, a new species from the rio São Francisco basin, Brazil (Ostariophysi: Characiformes: Characidae). Neotrop Ichthyol v.5, p. 237-244

Lunardelli B, Cabral MT, Vieira CED, Oliveira LF, Risso WE, Meletti PC, Martinez CBR. 2018. Chromium accumulation and biomarker responses in the Neotropical fish *Prochilodus lineatus* caged in a river under the influence of tannery activities. Ecotox Environ Safe 153, 188–194. doi:10.1016/j.ecoenv.2018.02.023

Mani R, Meena B, Valivittan K. 2014. Metallothionein expression in marine catfish *Arius arius* liver on exposure to cadmium using immunohistochemistry and western blot. Inter J Pharm Pharmaceutic Sci 6. 818-821

Mukhopadhyay I, Saxena DK, Chowdhuri DK. 2003. Hazardous effects of effluent from the chrome plating industry: 70 kDa heat shock protein expression as a marker of cellular damage in transgenic *Drosophila melanogaster* (Hsp70-lacZ). Environ Health Perspect v.111, p. 1926-1932

Nai GA, Golghetto GMS, Estrella MPS, Teixeira LDS, Moura FDC, Neto HB, Parizi JLS. 2015. The influence of water pH on the genesis of cadmium-induced câncer in a rat model. Histol Histopathol 30, 61-67

Nunes DMF, Magalhães ALB, Weber AA, Gomes RZ, Normando FT, Santiago KB, Bazzoli N. 2015. Influence of a large dam and importance of an undammed tributary on the reproductive ecology of the threatened fish matrinxã *Brycon orthotaenia* Günther, 1864 (Characiformes: Bryconidae) in southeastern Brazil. Neotropic Ichthyol 13(2), 317–324. doi:10.1590/1982-0224-20140084

Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C. 2003. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochem Biophysic Res Com v. 304, p. 505-512

Paschoalini AL, Savassi LA, Arantes FP, Rizzo E, Bazzoli N. 2019. Heavy metals accumulation and endocrine disruption in *Prochilodus argenteus* from a polluted neotropical river. Ecotox Env Safe 169, 539–550. doi:10.1016/j.ecoenv.2018.11.047

Polat F, Akın S, Yıldırım A, Dal T. 2015. The effects of point pollutants-originated heavy metals (lead, copper, iron, and cadmium) on fish living in Yeşilırmak River, Turkey. Toxicol Ind Heal 32, 1438–1449. doi:10.1177/0748233714565709

Poleksic V, Lenhardt M, Jaric I, Djordjevic D, Gacic Z, Cvijanovic G, Raskovic B. 2010. Liver, gills, and skin histopathology and heavy metal content of the Danube sterlet (*Acipenser ruthenus*, Linnaeus, 1758). Environ Toxicol Chem, 29(3), 515–521. doi:10.1002/etc.82

Procópio MS, Ribeiro HJ, Pereira LA, Oliveira-Lopes GA, Santana-Castro AOC, Rizzo E, Sato Y, Castro-Russo R, Corrêa JD. 2014. Sex-response differences of immunological and histopathological biomarkers in gill of *Prochilodus argenteus* from a polluted river in southeast Brazil. Fish Shellfish Immunol 39, 108–117. doi:10.1016/j.fsi.2014.04.010

Pyle GG, Rajotte JW, Couture P. 2005. Effects of industrial metals on wild fish populations along a metal contamination gradient. Ecotox Env Safe 61(3), 287–312. doi:10.1016/j.ecoenv.2004.09.003

Querol MVM, Querol E, Gomes NNA. 2002. Fator de condição gonadal, índice hepatossomático e recrutamento como indicadores do período de reprodução de *Loricariichthys platymetopon* (Osteichthyes, Loricariidae), bacia do rio Uruguai médio, sul do Brasil. Iheringia, Série Zoologia, 92(3), 79-84. doi: 10.1590/S0073-47212002000300008

Rajeshkumar S, Mini J, Munuswamy N. 2013. Effects of heavy metals on antioxidants and expression of HSP70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, Chennai, India. Ecotox Env Safe v.98, p. 8-18

Rajeshkumar S, Liu Y, Ma J, Duan HY, Li X. 2017. Effects of exposure to multiple heavy metals on biochemical and histopathological alterations in common carp, *Cyprinus carpio*. Fish Shellfish Immunol doi: 10.1016/j.fsi.2017.08.013

Ratn A, Prasad R, Awasthi Y, Kumar M, Misra A, Trivedi SP. 2018. Zn^{2+} induced molecular responses associated with oxidative stress, DNA damage and histopathological lesions in liver and kidney of the fish, *Channa punctatus* (Bloch, 1793). Ecotox Env Safe 151, 10–20.doi:10.1016/j.ecoenv.2017.12.058

Ruttkay-Nedecky B, Nejdl L, Gumulec J, Zitka O, Masarik M, Eckschlager T, Kizek R. 2013. The Role of Metallothionein in Oxidative Stress. Inter J Mol Sci 14(3), 6044–6066. doi:10.3390/ijms14036044

Safe S and Krishnan V. 1995. Cellular and molecular biology of aryl hydrocarbon (Ah) receptor-mediated gene expression. Arch Toxicol Suppl 17:99

Salim F. 2015. Histopathological Effect of heavy metal on different organs of freshwater fish tissues from Garmat Ali River adjacent to Al- Najebyia Power Station. Kufa J Vet Med Sci (6)1, 141-153

Santolin CVA, Ciminelli VST, Nascentes CC, Windmöller CC. 2015. Distribution and environmental impact evaluation of metals in sediments from the Doce River Basin, Brazil. Environ Earth Sci 74(2), 1235–1248. doi:10.1007/s12665-015-4115-2

Sassi A, Annabi A, Kessabi K, Kerkeni A, Saïd K, Messaoudi I. 2010. Influence of high temperature on cadmium-induced skeletal deformities in juvenile mosquitofish (*Gambusia affinis*). Fish Physiol Biochem 36, 403–409. doi:10.1007/s10695-009-9307-9

Savassi LA, Arantes FP, Gomes MVT, Bazzoli N. 2016. Heavy Metals and Histopathological Alterations in *Salminus franciscanus* (Lima & Britski, 2007) (Pisces: Characiformes) in the Paraopeba River, Minas Gerais, Brazil. Bull Environ Contam Toxicol 478–483. doi:10.1007/s00128-016-1732-8

Sevcikova M, Modra H, Kruzikova K, Zitka O, Hynek D, Adam V. 2013. Effect of Metals on Metallothionein Content in Fish from Skalka and Zelivka Reservoirs Int. J. Electrochem Sci 8, pp. 1650-1663

Somasundaram S, Abraham J, Maurya S, Makhija S, Gupta R, Toteja R. 2018. Cellular and molecular basis of heavy metal-induced stress in ciliates. Current Sci 114. 10.18520/cs/v114/i09/1858-1865

Squadrone S, Prearo M, Brizio P, Gavinelli S, Pellegrino M, Scanzio T, Guarise S, Benedetto A, Abete MC. 2013. Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) fromItalian Rivers. Chemosphere 90, 358-65

Taweel A, Shuhaimi-Othman M, Ahmad AK. 2013. Assessment of heavy metals in tilapia fish (*Oreochromis niloticus*) from the Langat river and Engineering lake in Bangi, Malaysia, and evaluation of health risk from tilapia consumption. Ecotox Env Safe 93, 45–51

Thévenod F and Lee WK. 2015. Live and Let Die: Roles of Autophagy in Cadmium Nephrotoxicity. Toxics v.3, p. 130-151

Vieira C, Morais S, Ramos S, Delerue-Matos C, Oliveira MBPP. 2011. Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra- and inter-specific variability and human health risks for consumption. Food Chem Toxicol 49, 923-932

Weber AA, Nunes DMF, Gomes RZ, Rizzo E, Santiago KB, Bazzoli N. 2013. Downstream impacts of a dam and influence of a tributary on the reproductive success of *Leporinus reinhardti* in São Francisco River. Aquatic Biol 19, 195-200

Weber AA, Moreira DP, Melo RMC, Vieira ABC, Prado PS, Silva MAN, Bazzoli N, Rizzo E. 2017. Reproductive effects of oestrogenic endocrine disrupting chemicals in *Astyanax rivularis* inhabiting headwaters of the Velhas River, Brazil. Sci Total Environ 592, 693–703. doi:10.1016/j.scitotenv.2017.02.181

WHO - World Health Organization. 2002. Codex Alimentarius - general standards for contaminants and toxins in food Schedule maximum and guideline levels for contaminants and toxins in food. Reference CX/FAC 02/16. Joint FAO/WHO Food Standards Programme, Codex Committee, Rotterdam, The Netherland.

Wilhelm S, Henneberg A, Köhler HR, Rault M, Richter D, Scheurer M, Triebskorn R. 2017. Does wastewater treatment plant upgrading with activated carbon result in an improvement of fish health? Aquatic Toxicol 192, 184–197. doi:10.1016/j.aquatox.2017.09.017

Zhong W, Zhang Y, Wu Z, Yang R, Chen X, Yang J, Zhu L. 2018. Health risk assessment of heavy metals in freshwater fish in the central and eastern North China. Ecotox Env Safe 157, 343–349. doi:10.1016/j.ecoenv.2018.03.048

Figures

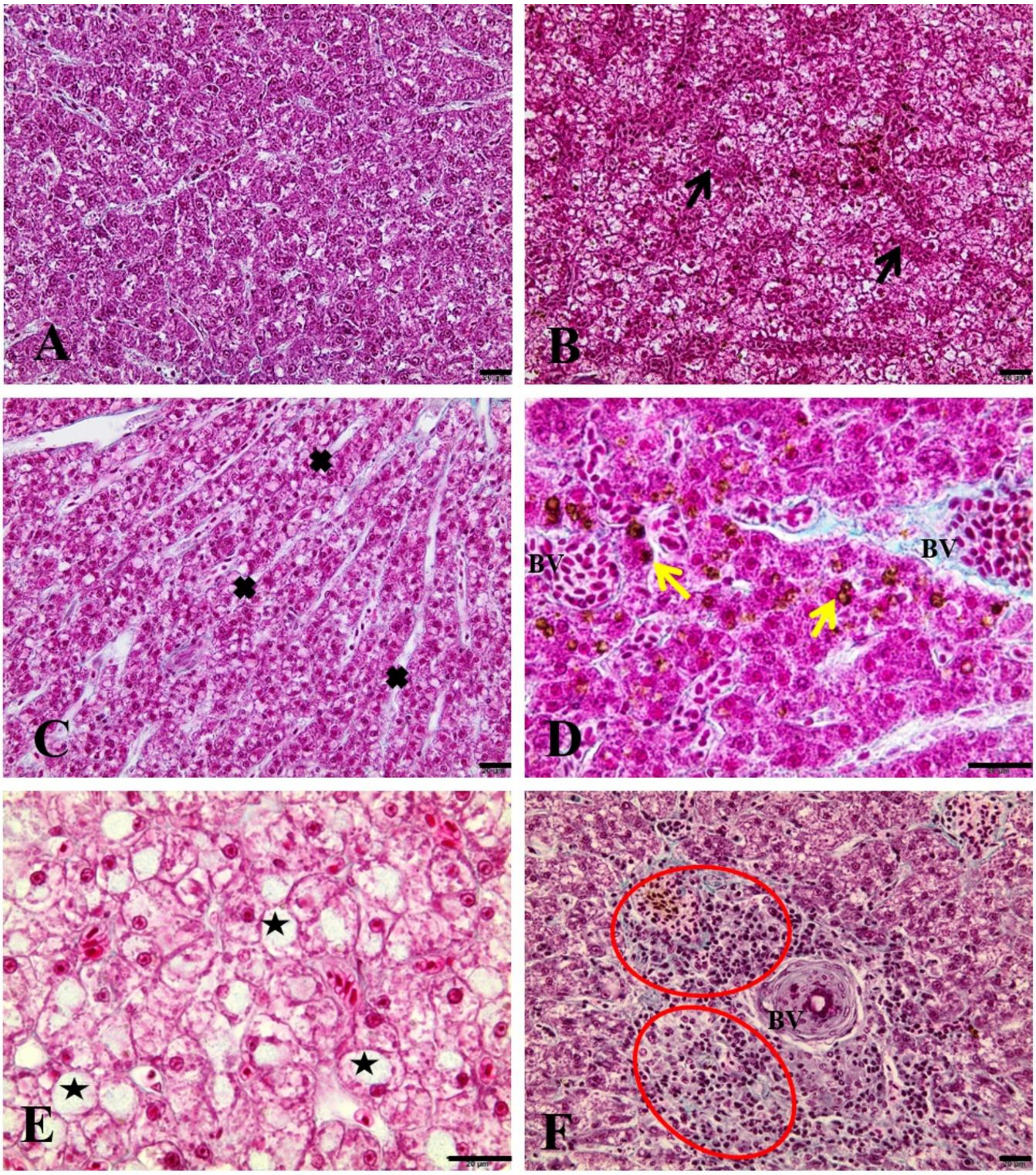


Figure 1. Histopathological assessment of the liver of *S. franciscanus* from Abaeté and Paraopeba rivers, São Francisco River Basin, Brazil. (A) Liver tissue showing normal morphology of a fish from the Abaeté River (200x). B, C, D, E and F represent histopathological alterations in fish from the Paraopeba River: (B) congestion of the sinusoid capillaries (black arrow) (200x); (C) dilatation of the sinusoid capillaries (black cross) (200x); (D) accumulation of pigmented macrophages (yellow arrow), aggregated among blood vessels (bv) (400x); (E) cytoplasmic vacuolization with nuclear displacement (black star) (400x); (F) infiltration of immune cells (red circle) next to blood vessels (bv) (100x). Scale bar = 20μm.

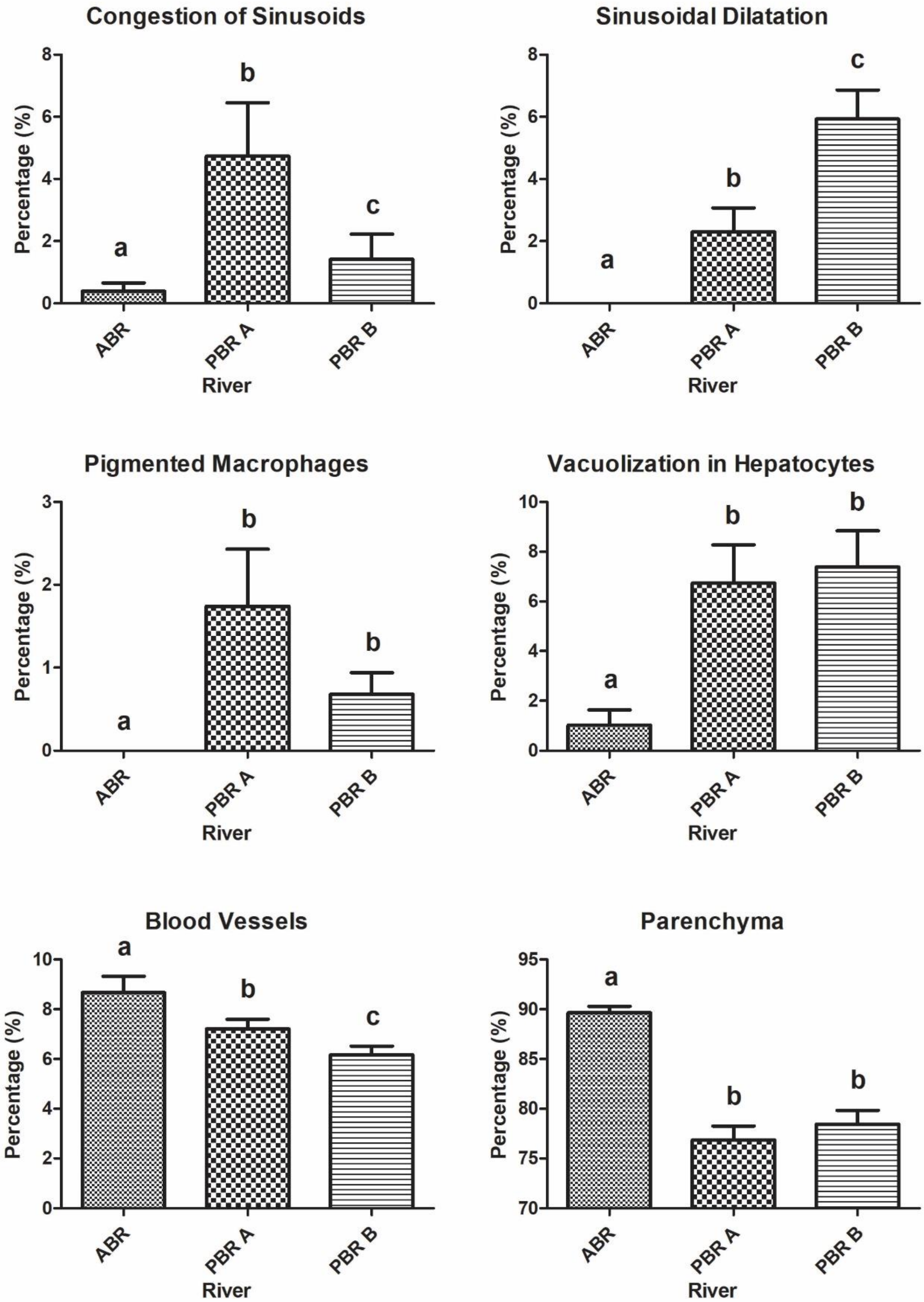


Figure 2. Quantification of histopathological alterations detected in liver of *S. francicanus* captured in the Abaeté (ABR) and Paraopeba (PBR A and PBR B) rivers, São Francisco River basin, Brazil. Different letters indicate statistical differences between the sampled sites ($p<0.05$; Kruskal-Wallis test).

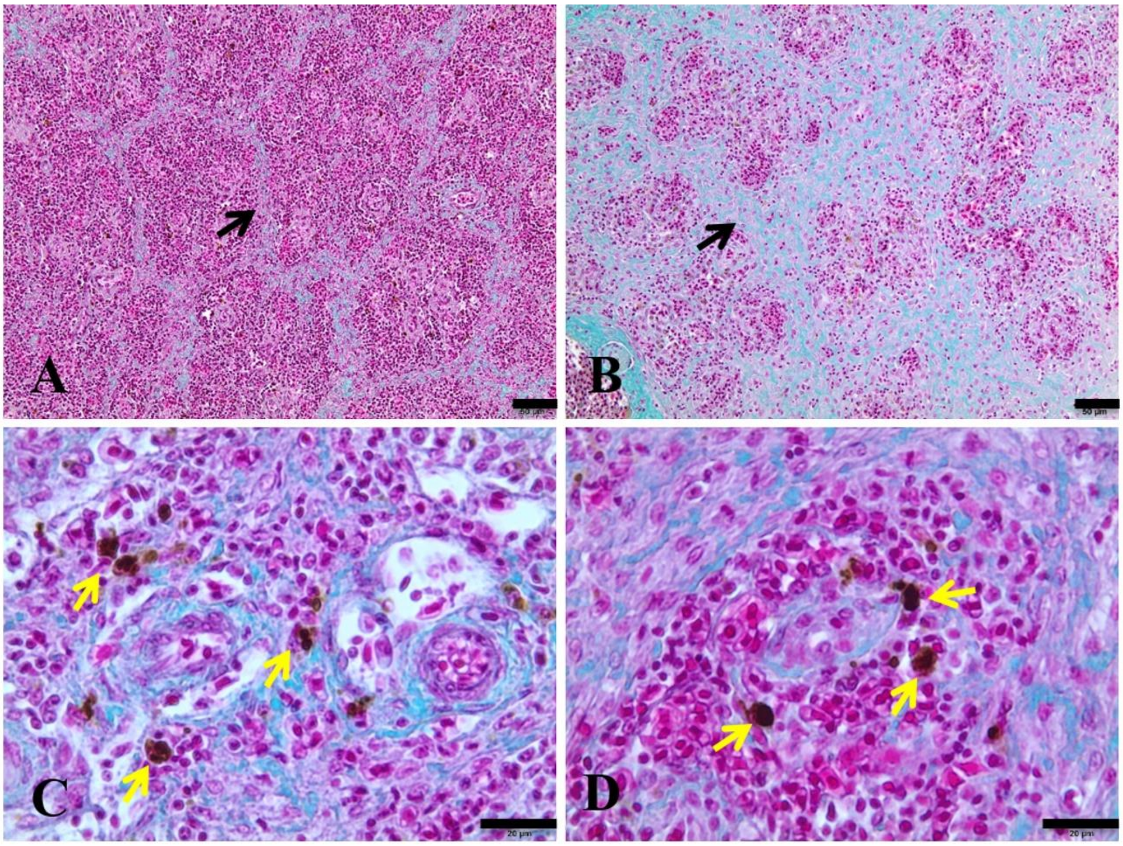


Figure 3. Histopathological assessment of the spleen of *S. franciscanus* from the Abaete and Paraopeba rivers, São Francisco River basin, Brazil. (A) Normal aspect of splenic tissue. Normal distribution between the stromal area (stained in green, identified by the black arrow) and the parenchymal area (stained in pink / purple) for fish from the Abaeté River (100x), scale bar = 50µm. B, C, and D represent alterations in the spleen of fish from Paraopeba River: (B) excessive connective tissue production, denominated fibrosis (100x), scale bar = 50µm; (C and D) accumulation of pigmented macrophages, distributed in parenchymal area (yellow arrow), scale bar = 20µm (400x).

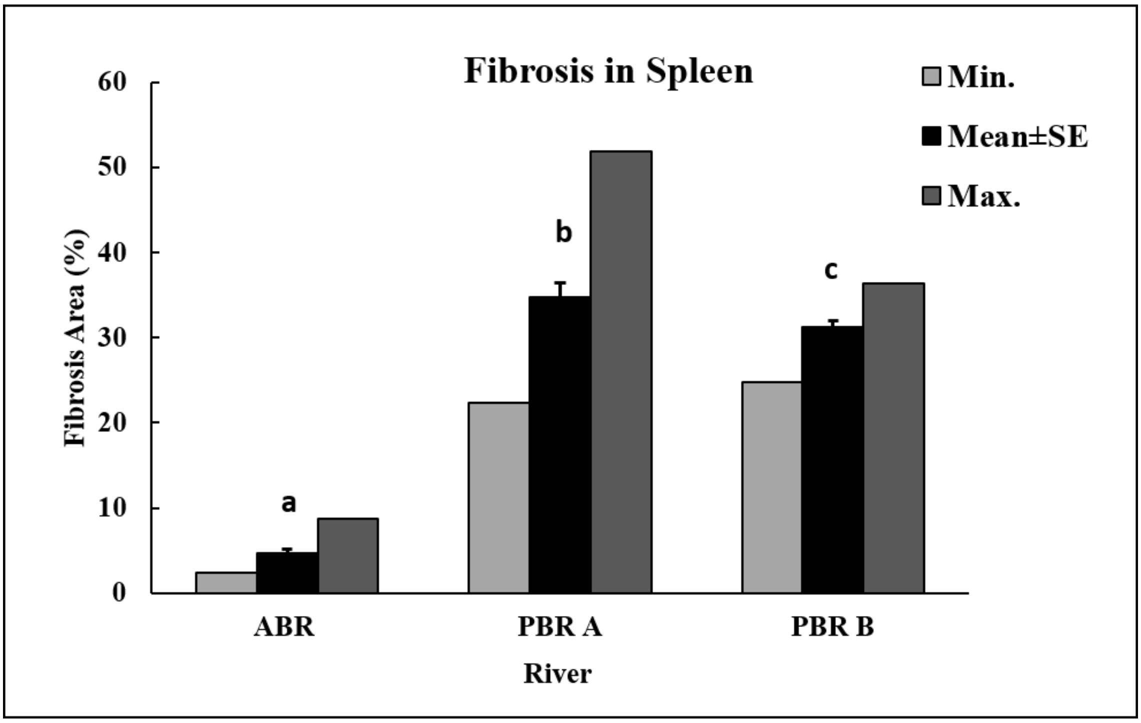


Figure 4. Quantification of fibrosis area in the spleen of *S. franciscanus* collected in the Abaeté (ABR) and Paraopeba (sites PBR A and PBR B) rivers. Different letters indicate statistical differences between sampled sites ($p<0.05$; Kruskal-Wallis test).

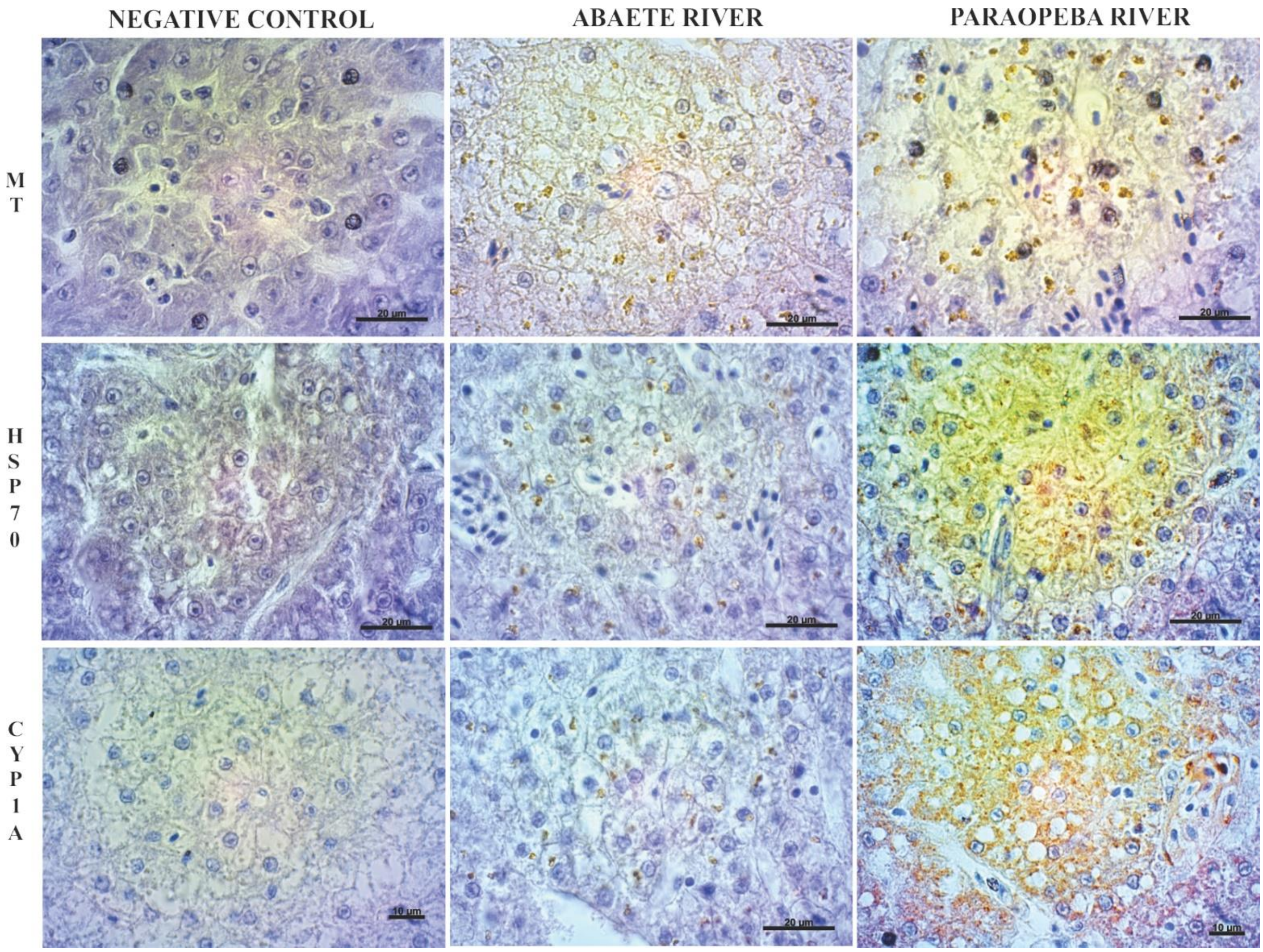


Figure 5. Immunohistochemical reactions of biomarkers in liver of *S. franciscanus*, from Abaeté and Paraopeba rivers: metallothionein (MT), heat shock protein 70 (HSP70) and cytochrome P4501A (CYP1A).

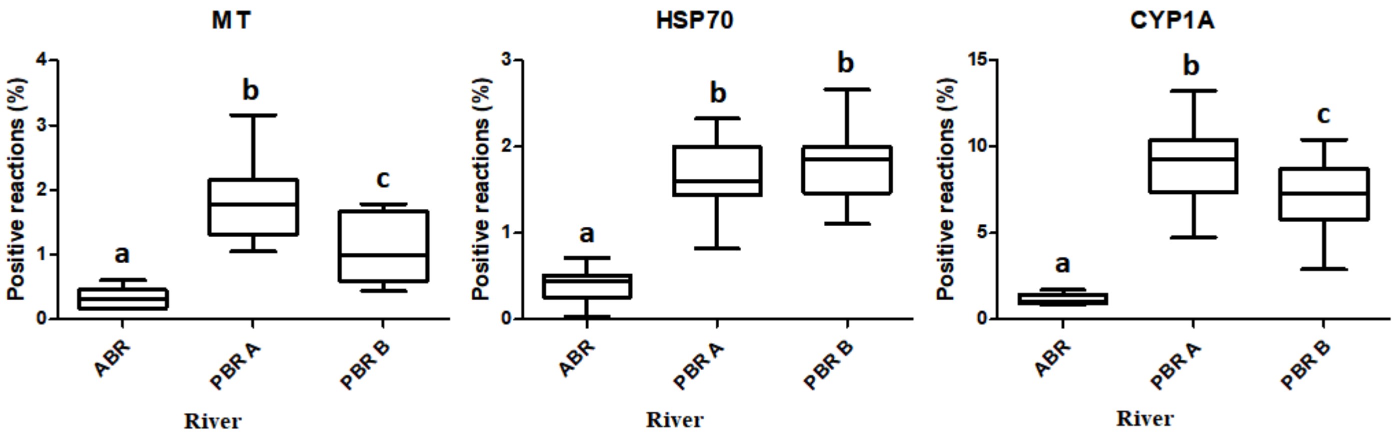
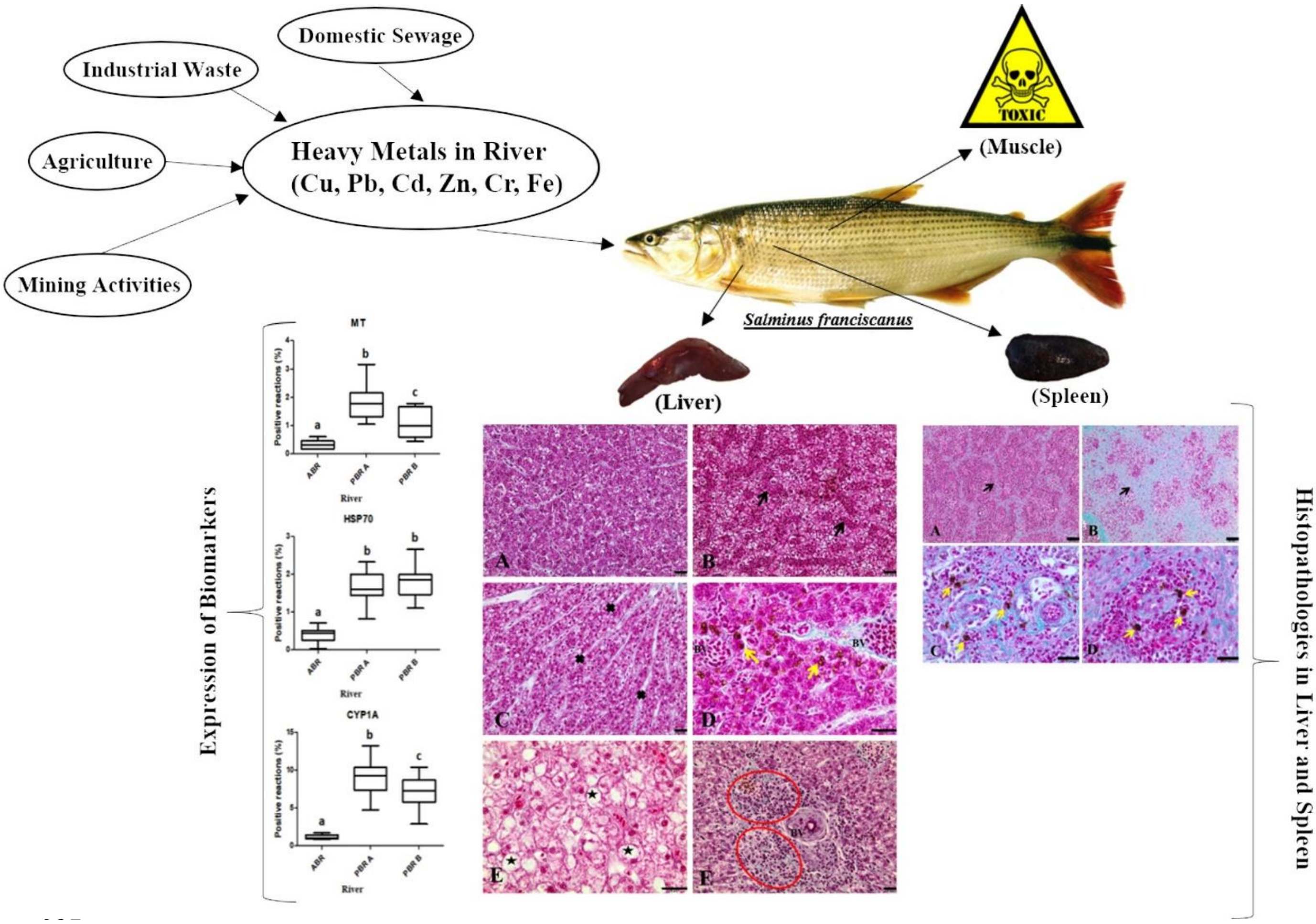


Figure 6. Quantification of positive reactions to immunohistochemistry peroxidase for metallothionein (MT), heat shock protein 70 (HSP70) and cytochrome P4501A (CYP1A) in liver of *S. franciscanus* from the Abaeté (ABR) and Paraopeba (sites PBR A and PBR B) rivers. Different letters indicate statistical differences between sampled sites ($p<0.05$; Kruskal-Wallis test).



Graphical Abstract: Heavy metals contamination, from diverse polluting sources, are bioaccumulated by fish and cause severe histopathologies, in target organs, including differences on expression of immunohistochemical environmental biomarkers, which will protect the organism against injury resulting from this environmental chemical pollution.

Tables

Table 1 - Physico-chemical parameters of the water of Abaeté and Paraopeba Rivers (Sites ABR, PBR A and PBR B), São Francisco River Basin, Brazil, for the years 2010, 2011 and 2012.

Sites	Oxygen	Temperature	pH	Conductivity
ABR	7.07 ± 1.07^{A}	26.34 ± 0.47^{A}	7.02 ± 0.14^{A}	64.67 ± 2.54^{A}
PBR A	7.25 ± 0.3^{A}	24.64 ± 1.03^{A}	6.77 ± 0.14^{A}	84.12 ± 6.07^{B}
PBR B	7.13 ± 0.25^{A}	25.09 ± 0.78^{A}	6.89 ± 0.16^{A}	77.67 ± 4.86^{B}

Data from Minas Gerais Institute of Water Management (IGAM, 2012[B]). Different letters indicate statistical differences between the sampling sections ($p<0.05$).

Table 2 – Heavy metal contamination of the water of Abaeté and Paraopeba Rivers (Sites ABR, PBR A and PBR B), São Francisco River basin, Brazil, for the years 2010, 2011 and 2012.

Sites	Heavy Metals (mg/ml)					
	Cu	**Pb**	**Cd**	**Zn**	**Cr**	**Fe**
ABR	nd	nd	nd	0.005 ± 0.001^{a}	nd	0.1 ± 0.05^{a}
PBR A	nd	0.002 ± 0.001^{a}	nd	0.07 ± 0.01^{b}	0.04 ± 0.01^{a}	1.32 ± 0.05^{b}
PBR B	nd	0.002 ± 0.001^{a}	0.0005	0.02 ± 0.01^{b}	0.04 ± 0.01^{a}	0.8 ± 0.05^{c}
Water limits CCME (2011)	0.002	0.001	0.00009	0.03	0.001	0.3

Data obtained by IGAM in October to February 2010, 2011 and 2012. Values are expressed as mean±S.E Different letters indicate statistical differences between the sampling sections ($p < 0.05$). nd = not detected.

Table 3 - Biometric data and biological indices for male and female fish of *Salminus franciscanus* from Abaeté and Paraopeba Rivers (Sites ABR, PBR A and PBR B), São Francisco River Basin, Brazil.

Sex	Sites	TL(cm)	BW(g)	GSI(%)	HSI(%)	K
Males	*ABR*	61.5 ± 13.9^{a}	2822.4 ± 1550.7^{a}	1.27 ± 0.76^{a}	0.64 ± 0.77^{a}	1.06 ± 0.07^{a}
	PBR A	65.0 ± 6.6^{a}	3316.6 ± 1224.2^{a}	1.01 ± 0.58^{a}	0.77 ± 0.15^{b}	1.18 ± 0.10^{b}
	PBR B	63.0 ± 8.3^{a}	2975.1 ± 1203.4^{a}	0.85 ± 0.15^{b}	0.70 ± 0.07^{b}	1.27 ± 0.11^{b}
Females	*ABR*	77.5 ± 5.7^{a}	5744.9 ± 1270.0^{a}	5.07 ± 1.85^{a}	0.77 ± 0.15^{a}	1.24 ± 0.11^{a}
	PBR A	79.0 ± 13.0^{a}	7353.3 ± 1603.0^{a}	3.03 ± 0.93^{b}	0.90 ± 0.18^{b}	1.47 ± 0.17^{b}
	PBR B	73.06 ± 3.7^{a}	5253.8 ± 848.9^{a}	2.52 ± 0.11^{b}	0.84 ± 0.12^{b}	1.41 ± 0.07^{b}

Different letters indicate statistical differences between the sampling sections ($p<0.05$).

Table 4 - Concentrations of heavy metals in liver and muscle of *Salminus franciscanus* from Abaeté and Paraopeba Rivers (Sites ABR, PBR A and PBR B), São Francisco River Basin, Brazil.

	Heavy metals (mg/kg)					
	Cu	**Pb**	**Cd**	**Zn**	**Cr**	**Fe**
Abaeté River						
Liver	19.12 ± 4.52^{a}	0.18 ± 0.3^{a}	0.07 ± 0.06^{a}	19.54 ± 4.31^{a}	0.65 ± 0.15^{a}	90.92 ± 14.53^{a}
Muscle	5.45 ± 0.41^{a}	0.12 ± 0.08^{a}	0.02 ± 0.01^{a}	8.88 ± 1.55^{a}	0.56 ± 0.12^{a}	7.13 ± 1.47^{a}
Paraopeba River - site A						
Liver	108.58 ± 22.42^{b}	2.28 ± 0.21^{b}	0.43 ± 0.05^{b}	173.87 ± 35.54^{b}	1.77 ± 0.08^{b}	1639.24 ± 502.09^{b}
Muscle	32.20 ± 0.21^{b}	2.24 ± 0.24^{b}	0.37 ± 0.02^{b}	13.40 ± 2.39^{b}	1.73 ± 0.10^{b}	210.15 ± 41.27^{b}
Paraopeba River - site B						
Liver	71.49 ± 15.70^{c}	1.42 ± 0.11^{b}	0.33 ± 0.22^{b}	160.48 ± 30.07^{b}	0.48 ± 0.23^{a}	1028.23 ± 388.59^{c}
Muscle	18.40 ± 5.66^{c}	0.73 ± 0.07^{c}	0.25 ± 0.03^{b}	27.84 ± 9.54^{c}	0.24 ± 0.04^{a}	186.68 ± 32.68^{b}
Tissue limits (mg/kg)						
OMS (2002)	30.0	0.2	0.05	nf	nf	109.0
ANVISA (2013)	30.0	0.3	0.05	50.0	0.1	nf

Data are expressed as mean ± SEM. Different letters indicate statistical differences between the sampling sections ($p<0.05$) / nf = not found. Copper (Cu), Lead (Pb), Cadmium (Cd), Zinc (Zn), Chromium (Cr) and Iron (Fe).

5. CONSIDERAÇÕES FINAIS

O presente estudo fornece informações suficientes para atestar que metais pesados foram detectados em concentrações elevadas na musculatura dos peixes do rio Paraopeba (Cu, Pb, Cd, Cr), excedendo os limites seguros de contaminantes em alimentos, estabelecido pela Agência Nacional de Vigilância Sanitária (ANVISA, 2013). Em órgãos de intenso metabolismo, tal como fígado, essa concentração de metais pesados (Cu, Pb, Cd, Zn, Cr e Fe, todos os metais analisados) foram registradas em valores muito acima dos permitidos pela ANVISA também, indicando o papel do fígado em bioacumular e metabolizar esses elementos, assim justificando a importância de estudos toxicológicos e seus efeitos sobre o organismo. A bioacumulação de metais pesados no *Salminus franciscanus* está relacionada à maior ocorrência de alterações histopatológicas no fígado e baço dos peixes, expostos a esta classe de contaminantes. A expressão de proteínas biomarcadoras de impacto ambiental tais como Metalotioneínas (MT), Proteínas do Choque Térmico (HSP70) e Citocromo P450 (CYP1A) se mostraram eficazes na avaliação de ambientes contaminados por metais pesados, e quando utilizadas em conjunto à outras ferramentas de avaliação ambiental apresentam uma maior confiabilidade e eficiência na avaliação dos impactos ambientais. As alterações histopatológicas, associadas às elevações significativas na expressão de MT, HSP70 e CYP1A no fígado dos peixes, sugerem o importante papel da utilização destes biomarcadores na avaliação da qualidade ambiental em ecossistemas contaminados por metais pesados, embora os estudos sobre o tema ainda sejam escassos. Em suma, os dados obtidos no presente estudo, evidenciam que metais pesados podem influenciar negativamente a biologia de *Salminus franciscanus* e à saúde humana, através do consumo de peixes contaminados pela população.

6. ANEXO I

- Participação na coautoria do seguinte trabalho recentemente publicado (Paschoalini et al., 2019):

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Heavy metals accumulation and endocrine disruption in *Prochilodus argenteus* from a polluted neotropical river

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ABSTRACT

Heavy metals are considered major pollutants of aquatic environments due to the difficulty of metabolization and the bioaccumulative potential in tissues of aquatic organisms, especially fish muscle that is often used as food worldwide. In addition to causing cell damage, some metals such as aluminium (Al), cadmium (Cd), copper (Cu), and lead (Pb) can act as endocrine disrupting chemicals in fish. The Paraopeba and Abaete Rivers are important tributaries of the upper São Francisco River basin, but the Paraopeba River receives, along its course, the discharge of many types of effluents that affect fish species, including widely consumed species such as *Prochilodus argenteus*. This study evaluated histological and molecular changes caused by chronic exposure to heavy metals in *P. argenteus* from the Paraopeba River and compared this to fish from the non-impacted Abaete River. Sampled fish from both rivers were used in histological analyses and immunohistochemical assays. The results showed increased incidence of histopathologies and changes in number and morphology of germline cells in both sexes. In addition, up-regulated expression of oestrogens-induced proteins in the liver of males were detected in polluted environment. All the alterations were related to the concentration of metals in water and fish. The high concentration of various metals observed in water and fish from Paraopeba River serves as an alert to the environmental and public health regulatory authorities.

7. REFERÊNCIAS BIBLIOGRÁFICAS

Ali, H. & Khan, E. 2018. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—Concepts and implications for wildlife and human health. Human and Ecological Risk Assessment: An International Journal, 1–24. doi:10.1080/10807039.2018.1469398

Arantes, F.P., Santos, H.B., Rizzo, E., Sato, Y., Bazzoli, N., 2010. Profiles of sex steroids, fecundity, and spawning of the curimatã-pacu *Prochilodus argenteus* in the São Francisco River, downstream from the Três Marias Dam, Southeastern Brazil. Animal Reproduction Science 118, 330-336.

Arantes, F.P., Savassi, L.A., Santos, H.B., Gomes, M.V.T., Bazzoli, N., 2016. Bioaccumulation of mercury, cadmium, zinc, chromium, and lead in muscle, liver, and spleen tissues of a large commercially valuable catfish species from Brazil. An. Acad. Bras. Cienc. 88, 137–147. doi:10.1590/0001-3765201620140434.

Atli, G., & Canli, M., 2010. Response of antioxidant system of freshwater fish Oreochromis niloticus to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. Ecotoxicology and Environmental Safety, 73(8), 1884–1889. doi:10.1016/j.ecoenv.2010.09.005

Barone, G., Stuffler, R.G., Storelli, M., 2013. Comparative study on trace metal accumulation in the liver of two fish species (Torpedinidae): Concentration-size relationship. Ecotoxicology and Environmental Safety. 97, 73-77.

Cao, X., Bi, R., Song, Y., 2017. Toxic responses of cytochrome P450 sub-enzyme activities to heavy metals exposure in soil and correlation with their bioaccumulation in *Eisenia fetida*. Ecotoxicology and Environmental Safety, 144, 158–165. doi:10.1016/j.ecoenv.2017.06.023

Carvalho, C.S., Araújo, H.S.S., Fernandes, M.N., 2004. Hepatic metallothionein in a teleost (*Prochilodus scrofa*) exposed to cooper at pH 4.5 and pH 8.0. Comparative Biochemistry and Physiology. Part B. v. 137, p. 225-234.

Castro-González, M.I. and Méndez-Armenta, M., 2008. Heavy metals: Implications associated to fish consumption. Environmental Toxicology & Pharmacology. 26, 263-271.

Chan, K.M., 1995. Metallothionein: potential biomarker for monitoring heavy metal pollution in fish around Hong Kong. Marine Pollution Bulletin, v. 31, p. 411-415.

D'costa, A., Shyama, S.K., Kumar, P., 2017. Bioaccumulation of trace metals and total petroleum and genotoxicity responses in an edible fish population as indicators of marine pollution. Ecotoxicology and environmental safety. 142. 22-28. 10.1016/j.ecoenv.2017.03.049

De la Torre, F.R., Salibián, A., Ferrari, L., 2007. Assessment of the pollution impact on biomarkers of effect of freshwater fish. Chemosphere, v. 68, p. 1582-1590.

El-Shehawi, A.M., Ali, F.K., Seehy, M.A., 2007. Estimation of water pollution by genetic biomarkers in tilapia and catfish species shows species-site interaction. African Journal of Biotechnology, v. 6, p. 840-846.

Feder, M.E. and Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones and the stress response: evolutionary and ecological physiology. Annual Reviews of Physiology, v. 61, p. 243-282.

Fergusson, J.E., 1990. The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Oxford: Pergamon Press.

Fishelson, L., 2006. Cytomorphological alterations of the thymus, spleen, head-kidney, and liver in cardinal fish (Apogonidae, Teleostei) as bioindicators of stress. Journal of Morphology. 267, 57-69.

Freitas, L.J.A, Prado, P.S., Arantes, F.P., Santiago, K.B., Sato, Y., Bazzoli, N., Rizzo, E., 2013. Reproductive biology of the characid dourado *Salminus franciscanus* from the São Francisco River, Brasil. Animal Reproduction Science. 139, 145–154.

Gomes, M.V.T. & Sato, Y., 2011. Avaliação da contaminação por metais pesados em peixes do rio São Francisco à jusante da represa de Três Marias, Minas Gerais, Brasil. Saúde & Ambiente em Revista. 6, 24-30.

Hossain, M. B., Ahmed, A.S.S., Sarker, M.S.I. 2018. Human health risks of Hg, As, Mn, and Cr through consumption of fish, Ticto barb (*Puntius ticto*) from a tropical river, Bangladesh. Environmental Science and Pollution Research. doi:10.1007/s11356-018-3158-9

IGAM[a] (Instituto mineiro de gestão das águas), 2012. Estudos das Metas de Qualidade da Bacia Hidrográfica do Rio Paraopeba. Avaliable in: http://comites.igam.mg.gov.br/comites-estaduais/bacia-do-rio-sao-francisco/sf3-cbh-do-rio-paraopeba/1104-conheca-a-bacia. Acess in march / 2015.

IGAM[b] (Instituto Mineiro de Gestao das Aguas), 2012. Monitoramento da qualidade das águas superficiais no estado de Minas Gerais, Brasil. Quarterly report avaliable in: http://www.igam.mg.gov.br/images/ stories/ qualidade/2013/relatorio-trimestral-am-3o-trim-2012.pdf.

Janz, D.M., Mc Master, M.E., Weber, L.P., Munkittrick, K.R., Kraak, G.V.D., 2001. Recovery of ovary size, follicle cell apoptosis, and HSP70 expression in fish exposed to bleached pulp mill effluent. Canadian Journal of Fisheries and Aquatic Sciences, v. 58, p. 620-625.

Jia, Y., Wang, L., Cao, J., Li, S., Yang, Z. 2018. Trace elements in four freshwater fish from a mine-impacted river: spatial distribution, species-specific accumulation, and risk assessment. Environmental Science and Pollution Research, 25(9), 8861–8870. doi:10.1007/s11356-018-1207-z

Jiang, Z., Xu, N., Liu, B., Zhou, L., Wang, J., Wang, C., Xiong, W., 2018. Metal concentrations and risk assessment in water, sediment and economic fish species with various habitat preferences and trophic guilds from Lake Caizi, Southeast China. Ecotoxicology and Environmental Safety, 157, 1–8. doi:10.1016/j.ecoenv.2018.03.078

Kawajiri, Kaname & Fujii-Kuriyama, Yoshiaki., 2007. Cytochrome P450 gene regulation and physiological functions mediated by the aryl hydrocarbon receptor. Archives of biochemistry and biophysics. 464. 207-12. 10.1016/j.abb.2007.03.038

Knapen, D., Reynders, H., Bervoets, L., Verheyen, E., Blust, R., 2007. Metallothionein gene and expression as a biomarker for metal pollution in natural gudgeon populations. Aquatic Toxicology, v. 82, p. 163-172.

Kwok, J., Liang, Y., Wang, H., Dong, Y.H., Leung, S.Y., Wong, M., 2014. Bioaccumulation of heavy metals in fish and Ardeid at Pearl River Estuary, China. Ecotoxicology and environmental safety. 106C. 62-67. 10.1016/j.ecoenv.2014.04.016

Li, S., Tan, H.-Y., Wang, N., Zhang, Z.-J., Lao, L., Wong, C.-W., & Feng, Y., 2015. The Role of Oxidative Stress and Antioxidants in Liver Diseases. International Journal of Molecular Sciences, 16(11), 26087–26124. doi:10.3390/ijms161125942

Lima, F.C.T. & Britski, H.A., 2007. *Salminus franciscanus*, a new species from the rio São Francisco basin, Brazil (Ostariophysi: Characiformes: Characidae). Neotropical Ichthyology, v. 5, p. 237-244.

Liu, J., Cao, L., Dou, S. 2019. Trophic transfer, biomagnification and risk assessments of four common heavy metals in the food web of Laizhou Bay, the Bohai Sea. Science of The Total Environment. doi:10.1016/j.scitotenv.2019.03.140

Łuczyńska, J., Paszczyk, B., Łuczyński, M.J., 2018. Fish as a bioindicator of heavy metals pollution in aquatic ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health. Ecotoxicology and Environmental Safety, 153, 60–67. doi:10.1016/j.ecoenv.2018.01.057

Lunardelli, B., Cabral, M.T., Vieira, C.E.D., Oliveira, L.F., Risso, W.E., Meletti, P.C., Martinez, C.B.R., 2018. Chromium accumulation and biomarker responses in the Neotropical fish *Prochilodus lineatus* caged in a river under the influence of tannery activities. Ecotoxicology and Environmental Safety, 153, 188–194. doi:10.1016/j.ecoenv.2018.02.023

Marijic, V.F. & Raspor, B., 2006. Age and tissue-dependent metallothionein and cytosolic metal distribuition in a native Mediterranean fish *Mullus barbatus*, from the Eastern Adriatic Sea. Comparative Biochemistry and Physiology. Part C, v. 143, p. 382-387.

Marques, D.C., Matta, S.L.P., Oliveira, J.A., Dergam, J.A. 2009. Alterações histológicas em brânquias de *Astyanax bimaculatus* causadas pela exposição aguda ao zinco. Revista Brasileira de Toxicologia. 22, 26-26.

Masindi, V., & Muedi, K. L., 2018. Environmental Contamination by Heavy Metals. Intech Open, Chapter 7, 115-133. DOI: 10.5772/intechopen.76082

Mendil, D., 2005. Determination of trace metal levels in seven fish species in lakes in Tokat, Turkey. Food Chemistry. 90, 175-179.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. doi:10.1016/S1360-1385(02)02312-9

Monferran, M., Garnero, L.P., Wunderlin, D.A., Bistoni, M., 2016. Potential human health risks from metals and As via *Odontesthes bonariensis* consumption and ecological risk assessments in a eutrophic lake. Ecotoxicology and Environmental Safety. 129. 302-310. 10.1016/j.ecoenv.2016.03.030

Mosser, D.D., Caron, A.W., Bourget, L., Denis-Larose, C., Massie, B., 1997. Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. Mol Cell Biol, v. 17, p. 5317–5327.

Mukhopadhyay, I., Saxena, D.K., Chowdhuri D, K., 2003. Hazardous effects of effluent from the chrome plating industry: 70 kda heat shock protein expression as a marker of cellular damage in transgenic *Drosophila melanogaster* (Hsp70-lacZ). Environmental Health Perspectives, v. 111, p. 1926-1932.

Nai, G.A., Golghetto, G.M.S., Estrella, M.P.S., Teixeira, L.D.S., Moura, F.D.C., Neto, H.B., Parizi, J.L.S., 2015. The influence of water pH on the genesis of cadmium-induced câncer in a rat model. Histology and Histopathology. 30, 61-67.

Nunes, D.M.F., Magalhães, A.L.B., Weber, A.A., Gomes, R.Z., Normando, F.T., Santiago, K.B., Bazzoli, N., 2015. Influence of a large dam and importance of an undammed tributary on the reproductive ecology of the threatened fish matrinxã *Brycon orthotaenia* Günther, 1864 (Characiformes: Bryconidae) in southeastern Brazil. Neotropical Ichthyology, 13(2), 317–324. doi:10.1590/1982-0224-20140084

Parcellier, A., Gurbuxani, S., Schmitt, E., Solary, E., Garrido, C., 2003. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochemical and Biophysical Research Communications, v. 304, p. 505-512.

Paschoalini, A.L., Savassi, L.A., Arantes, F.P., Rizzo, E., Bazzoli, N., 2019. Heavy metals accumulation and endocrine disruption in *Prochilodus argenteus* from a polluted neotropical river. Ecotoxicology and Environmental Safety 169, 539–550. doi:10.1016/j.ecoenv.2018.11.047

Petrere., Jr.M., 1989. River fisheries in Brazil: a review. Regulated rivers: Research and Managment. 4, 1-16.

Prado, P.S., Souza, C.C., Bazzoli, N., Rizzo, E., 2011. Reproductive disruption in lambari *Astyanax fasciatus* from a Southeastern Brazilian reservoir. Ecotoxicology and Environmental Safety. 74, 1879–1887.

Protano, C., Zinna, L., Giampaoli, S., Spica, V.R., Chiavarini, S., Vitali, M., 2014. Heavy Metal Pollution and Potential Ecological Risks in Rivers: A Case Study from Southern ItalyBull Environ Contam Toxicol. 92, 75–80.

Rajeshkumar, S., Mini, J., Munuswamy, N., 2013. Effects of heavy metals on antioxidants and expression of HSP70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, Chennai, India. Ecotoxicology and Environmental Safety, v. 98, p. 8-18.

Rajeshkumar, S., Liu, Y., Ma, J., Duan, H.Y., Li, X., 2017. Effects of exposure to multiple heavy metals on biochemical and histopathological alterations in common carp, *Cyprinus carpio* L., Fish and Shellfish Immunology. doi: 10.1016/j.fsi.2017.08.013.

Reis, A.B., 2009. Alterações do epitélio branquial e das lamelas de tilápias (*Oreochromis niloticus*) causadas por mudanças do ambiente aquático em tanques de cultivo intensivo. Pesquisa Veterinária Brasileira. 29, 303-311.

Ribeiro, E.V., Magalhães-Jr, A.P., Horn, A.H., Trindade, W.M., 2012. Metais pesados e qualidade da água do rio São Francisco no segmento entre Três Marias e Pirapora, M.G.: índice de contaminação. Geonomos. 20, 49-63.

Ruttkay-Nedecky, B., Nejdl, L., Gumulec, J., Zitka, O., Masarik, M., Eckschlager, T., Kizek, R., 2013. The Role of Metallothionein in Oxidative Stress. International Journal of Molecular Sciences, 14(3), 6044–6066. doi:10.3390/ijms14036044

Safe S, Krishnan V., 1995. Cellular and molecular biology of aryl hydrocarbon (Ah) receptor-mediated gene expression. Arch. Toxicol. Suppl. 17:99.

Santos, H.B., Rizzo, E., Bazzoli, N., Sato, Y., Moro, L., 2005. Ovarian regression and apoptosis in the South American teleost *Leporinus taeniatus* Lutken (Characiformes, Anostomidae) from the São Francisco Basin. Journal of Fish Biology. 67, 1446–1459.

Sato, Y., & Godinho, H.P., 2003. Migratory fishes of the São Francisco River. Pp. 195-232 in: J. Carosfeld, B. Harvey, C. Ross & A. Baer (eds). Migratory fishes of South America: biology, fisheries and conservation status. World Fisheries Trust/The World Bank/International Development Research Centre, Ottawa, 372 pp

Sato, Y. & Sampaio, E.V., 2005. A ictiofauna na região do alto São Francisco, com ênfase no reservatório de Três Marias. In: Nogueira, M.G., Henry, R., Jorcin, A., (Org.). Ecologia de reservatórios: impactos potenciais, ações de manejo e sistemas em cascata. São Carlos: Rima Editora. 251-274.

Savassi, L.A., Arantes, F.P., Gomes, M.V.T., Bazzoli, N., 2016. Heavy Metals and Histopathological Alterations in *Salminus franciscanus* (Lima & Britski, 2007) (Pisces: Characiformes) in the Paraopeba River, Minas Gerais, Brazil. Bull. Environ. Contam. Toxicol. 478–483. doi:10.1007/s00128-016-1732-8

Sevcikova, M., Modra, H., Kruzikova, K., Zitka, O., Hynek, D., Adam, V., 2013. Effect of Metals on Metallothionein Content in Fish from Skalka and Zelivka Reservoirs Int. J. Electrochem. Sci., 8, pp. 1650-1663.

Somasundaram, S., Abraham, J., Maurya, S., Makhija, S., Gupta, R., Toteja, R., 2018. Cellular and molecular basis of heavy metal-induced stress in ciliates. Current science. 114. 10.18520/cs/v114/i09/1858-1865

Souza, M.L.R., 2003. Processamento do filé da pele de tilá¬pia do Nilo (*Oreochromis niloticus*): aspectos tecnológicos, composição centesimal, rendimento, vida útil do filé defumado e teste de resistência da pele curtida. 169 f. Tese (Doutorado em Zootecnia) - Universidade Estadual Paulista, Jaboticabal.

Squadrone, S., Prearo, M., Brizio, P., Gavinelli, S., Pellegrino, M., Scanzio, T., Guarise, S., Benedetto, A., Abete, M.C., 2013. Heavy metals distribution in muscle, liver, kidney and gill of European catfish (*Silurus glanis*) from Italian Rivers. Chemosphere. 90, 358-65.

Stillman, M.J., 1995. Metallothioneins. Coordination Chemistry Reviews, v. 14, p. 461-511.

Subotić, S., Spasić, S., Višnjić-Jeftić, Ž., Hegediš, A., Krpo-Ćetković, J., Lenhardt, M., 2013. Heavy metal and trace element bioaccumulation in target tissues of four edible fish species from the Danube River (Serbia). Ecotoxicology and Environmental Safety. 98, 196-202.

Suedel B.C., Boraczek J.A., Peddicord R.K., Clifford P.A., Dillon T.M., 1994. Trophic Transfer and Biomagnification Potential of Contaminants in Aquatic Ecosystems. In: Ware G.W. (eds) Reviews of Environmental Contamination and Toxicology. Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews), vol 136. Springer, New York, NY

Tabinda, A.B., Bashir, S., Yasar, A., Hussain, M., 2013. Metals concentrations in the riverine water, sediments and fishes from River Ravi at Balloki headworks. Journal of Animal and Plant Sciences. 23, 76-84.

Taweel, A., Shuhaimi-Othman, M., Ahmad, A.K., 2013. Assessment of heavy metals in tilapia fish (*Oreochromis niloticus*) from the Langat river and Engineering lake inBangi, Malaysia, and evaluation of health risk from tilapia consumption. Ecotoxicology and Environmental Safety. 93, 45–51.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., Sutton, D. J., 2012. Heavy Metal Toxicity and the Environment. Molecular, Clinical and Environmental Toxicology, 133–164. doi:10.1007/978-3-7643-8340-4_6

Thévenod, F. & Lee, W.K., 2015. Live and Let Die: Roles of Autophagy in Cadmium Nephrotoxicity. Toxics, v. 3, p. 130-151.

Thomé, R.G., Santos, H.B., Arantes, F.P., Domingos, F.F.T., Bazzoli, N., Rizzo, E., 2009. Dual roles of autophagy during follicular atresia in fish ovary. Autophagy. 1, 117-226.

Viarengo, A., Burlando, B., Dondero, F., Marro, A., Fabbri, R., 1999. Metallothionein as a tool in biomonitoring programs. Biomarkers, v. 4, p. 455-466.

Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C., Oliveira, M.B.P.P., 2011. Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: intra- and inter-specific variability and human health risks for consumption. Food & Chemical Toxicology. 49, 923-932.

Vitek, T., Spurny, P., Mares, J., Zikova, A., 2007. Heavy metal contamination of the Loucka River water ecosystem, Acta Veterinaria Brno. 76, 149-154.

Wang, S. & Shi, X., 2001. Molecular mechanisms of metal toxicity and carcinogenesis. Mol Cell Biochem, 222:3–9.

Weber, A.A., Nunes, D.M.F., Gomes, R.Z., Rizzo, E., Santiago, K.B., Bazzoli, N., 2013. Downstream impacts of a dam and influence of a tributary on the reproductive success of *Leporinus reinhardti* in São Francisco River. Aquatic Biology. 19, 195-200.

Weber, L.P. & Janz, D.M., 2001. Effect of naphthoflavone and dimethylbenz[a]anthracene on apoptosis and HSP70 expression in juvenile channel catfish (*Ictalurus punctatus*) ovary. Aquatic Toxicology, v. 54, p. 39-50.

Yi, Y., Tang, C., Yi, T., Yang, Z., Zhang, S., 2017. Health risk assessment of heavy metals in fish and accumulation patterns in food web in the upper Yangtze River, China. Ecotoxicology and Environmental Safety, 145, 295–302. doi:10.1016/j.ecoenv.2017.07.022

Zhong, W., Zhang, Y., Wu, Z., Yang, R., Chen, X., Yang, J., Zhu, L., 2018. Health risk assessment of heavy metals in freshwater fish in the central and eastern North China. Ecotoxicology and Environmental Safety, 157, 343–349. doi:10.1016/j.ecoenv.2018.03.048