FREDERICO FERNANDES FERREIRA

METALS CONCENTRATION IN FRESHWATER FISH FROM DOCE RIVER AND THE INFLUENCES OF THE SAMARCO'S DAM COLLAPSE, MARIANA, BRAZIL

Thesis submitted to the Graduate Program in Ecology of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

Adviser: Carlos Frankl Sperber

Co-adviser: Jorge Abdala Dergam dos Santos

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Assent:

Frederico Fernandes Ferreira
Author

Carlos Frankl Sperber
Adviser

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A crítica válida presta um favor ao cientista.

Carl Sagan

ABSTRACT

FERREIRA, Frederico Fernandes, D.Sc., Universidade Federal de Viçosa, July, 2022. **Metals concentration in freshwater fish from Doce River and the influences of the Samarco's Dam collapse, Mariana, Brazil.** Adviser: Carlos Frankl Sperber. Co-adviser: Jorge Abdala Dergam dos Santos.

The *Rio Doce* basin is located in southeastern Brazil, in the states of Minas Gerais (MG) and Espírito Santo (ES). The basin is characterized by intense economic activity, which, added to the high population density and historical processes of extractivism, makes it one of the Brazilian drainages most impacted by human activities. With the disposal of domestic sewage, agricultural and industrial inputs in the channel of the Doce River and its tributaries, violations of water quality parameters such as total phosphate and fecal coliforms are historically recorded for the basin. The collapse of the SAMARCO dam threw 62 tons of tailings into the Doce River channel. Covering fish microhabitats, foraging and breeding areas, aggravating the state of degradation of the basin and modifying the physical and chemical characteristics of the river bed sediment. The tailings avalanche turned over the river bottom, resuspending the sediment and bio making available metals that were previously trapped in lower river layers. The composition of the tailings is mainly silica and iron oxides, but studies indicate high levels of other elements such as Aluminum, Arsenic and Manganese. Thus, the tailings contributed directly and indirectly to the mobilization of metals for the aquatic biota. Bioaccumulation within fish and biomagnification of metals in the food chain can generate irreversible impacts on aquatic biota given its persistence in biota, not being decomposed, increasing its toxicity. Our results indicate mercury biomagnification and bioaccumulation of several elements such as arsenic and mercury, for several of the studied species, and the result varies in an interspecific way. Some species are more subject to bioaccumulation, among them those with piscivorous and benthic habits stand out. These groups should be a priority target for monitoring the concentrations of metals in the ichthyofauna with a focus on food security and maintenance of fish communities in the basin. Among the tributaries studied, the Gualaxo do Norte and Carmo rivers deserve special attention, mainly due to the high mercury values recorded for the region's ichthyofauna. The historic gold extraction process in the region and the leaching and contamination of aquatic biota makes these tributaries the main target for monitoring and bioremediation studies.

Keywords: Fish. arsenic. Mercury. Bioaccumulation. Trophic level.

RESUMO

FERREIRA, Frederico Fernandes, D.Sc., Universidade Federal de Viçosa, julho de 2022. **Concentração de metais em peixes de água doce na bacia do Rio Doce e as influências do rompimento da barragem da Samarco, Mariana, Brasil.** Orientador: Carlos Frankl Sperber. Coorientador: Jorge Abdala Dergam dos Santos.

A bacia do Rio Doce está localizada no sudeste do Brasil, nos estados de Minas Gerais (MG) e Espírito Santo (ES). A bacia é caracterizada por intensa atividade econômica, que, somados à alta densidade populacional e aos processos históricos de extrativismo, a transforma em uma das drenagens brasileiras mais impactadas por atividades antrópicas. Com o descarte de esgoto doméstico, insumos agrícolas e industriais na calha do rio Doce e em seus afluentes, violações nos parâmetros da qualidade da água como fosfato total e coliformes fecais são historicamente registrados para a bacia. O rompimento da barragem da SAMARCO lançou 62 toneladas de rejeito para a calha do rio Doce, cobrindo microhabitats de peixes, áreas de forrageamento e reprodução, agravando o estado de degradação da bacia e modificando as características físicas e químicas do sedimento do leito do rio. A avalanche de rejeito revolveu o fundo do rio, ressuspendendo o sedimento e biodisponibilizando metais que antes estavam presos em camadas inferiores do rio. O rejeito é composto principalmente de sílica e óxidos de ferro, mas estudos indicam níveis elevados de outros elementos como Alumínio, Arsênio e Manganês. Assim, o rejeito contribuiu de forma direta e indireta para a mobilização de metais para a biota aquática. A bioacumulação dentro dos peixes e a biomagnificação de metais na cadeia alimentar pode gerar impactos irreversíveis para a biota aquática visto a sua persistência na biota, não sendo decomposto, o que aumenta sua toxicidade. Nossos resultados indicam biomagnificação de mercúrio e bioacumulação de vários elementos como arsênio e mercúrio, para várias das espécies estudadas, e o resultado varia de forma interespecífica. Algumas espécies estão mais sujeitas à bioacumulação, dentre elas destacam-se aquelas com hábitos piscívoros e bentônicos. Esses grupos devem ser alvo prioritário para o monitoramento das concentrações de metais na ictiofauna com foco na segurança alimentar e manutenção das comunidades pesqueiras da bacia. Dentre os afluentes estudados, os rios Gualaxo do Norte e Carmo merecem atenção especial, principalmente pelos altos valores de mercúrio registrados para a ictiofauna da região. O histórico processo de extração de ouro na região e a lixiviação e contaminação da biota

aquática fazem desses afluentes o principal alvo de estudos de monitoramento e biorremediação.

Palavras-chave: Peixes. Arsênio. Mercúrio. Bioacumulação. Nível trófico.

SUMÁRIO

INTRODUÇÃO GERAL

A água possui grande capacidade para dissolver quase todos os elementos e compostos químicos, sejam estes de origem natural, do solo, atmosfera, ou substâncias lançadas de forma inadequada pelo homem, causando poluição do ecossistema aquático (Martinez e Cólus 2002). A biota, inclusive a ictiofauna, é prejudicada pelo efeito danoso causado pelo lançamento de resíduos que modificam as características físicas, químicas e biológicas da água provocando problemas de eutrofização e toxicidade, com efeitos negativos sobre os peixes e sobre a própria qualidade da água (Camargo e Martinez 2006). Dentre os resíduos descartados de forma inadequada encontram-se os metais, que são considerados contaminantes críticos nos ecossistemas aquáticos pelo seu potencial de entrar no organismo, ser transferido, acumulando-se tanto no próprio organismo (bioacumulação) quanto à cadeia trófica (biomagnificação) (Webb et al 2015).

Os metais são importantes e parte integrante do ambiente e da matéria viva, ocorrendo naturalmente em pequenas concentrações, entre eles destacam-se o zinco (Zn), ferro (Fe), manganês (Mn), cobre (Cu) e o cobalto (Co) (Soetan et al 2010). Entretanto há metais que não são essenciais e possuem atividades deletérias no organismo (Ali et al 2019). Dentre eles o Hg, Cd, Pb, Cu, Zn, Co, Mn, alumínio (Al), arsênio (As), cromo (Cr), níquel (Ni), selênio (Se) e estanho (Sn), estes metais têm sido encontrados em altas concentrações em ambientes aquáticos (Rajaei et al 2012). Estes elementos reagem com macromoléculas e com ligantes difusores em membranas que conferem as propriedades de bioacumulação, biomagnificação na cadeia alimentar, persistência no ambiente e capacidade de provocar mudanças prejudiciais nos processos metabólicos dos seres vivos (Wood 2011). Mesmo aqueles metais que são essenciais para o organismo, em concentrações elevadas passam a ser prejudiciais (Watanabe et al 1997).

A bioacumulação de metais pode levar a concentrações altamente tóxicas para as diferentes espécies da biota, incluindo o ser humano. A persistência no ambiente gera os efeitos a longo prazo, mesmo depois de interrompidas as emissões (Ali et al 2019). Dependendo das concentrações atingidas no tecido do animal, os metais podem desnaturar proteínas, inativar enzimas, modificar a atividade celular, além de alterar as membranas celulares que irão causar a morte celular e destruição dos tecidos (Goldhaber 2003). Os efeitos de cada um desses metais são bem conhecidos nos seres humanos e são objeto de legislação específica pela ANVISA.

A ictiofauna da bacia do rio Doce inclui espécies com diferentes níveis tróficos e com relação variável com o substrato, oferecendo oportunidades relevantes para compreender o fluxo de metais na comunidade de peixes. Várias espécies se alimentam de itens próximos ao substrato, podendo ser migratórias, como as curimbas, *Prochilodus* spp., ou não migratórias como os acarás *Geophagus brasiliensis* e exóticas como as tilápias *Oreochromis* spp. além das espécies de cascudos como os *Hypostomus* spp. e *Loricariichthys castaneus*. Devido ao contato direto com o substrato, estas espécies podem ser mais impactadas pela presença do rejeito nas áreas afetadas.

Por outro lado, espécies de pequeno porte que se alimentam de vários itens, como as espécies de lambaris, apresentaram níveis de metais condizentes com o meio aquático e com o ambiente terrestre próximo aos cursos d'água. Algumas destas espécies apresentam curtos movimentos migratórios, outras espécies são menos vágeis e mostrarão as condições específicas de certos trechos dos afluentes e da calha (Gurgel 2004). O grupo dos piscívoros merece atenção especial, dada a sua maior condição de concentração de metais ao longo do tempo (Depew et al 2013; Johnson et al 2015). Na bacia do rio Doce são registradas várias espécies piscívoras de porte médio e grande como as *Hoplias* spp. e o exótico *Salminus brasiliensis*.

Diversas outras espécies exóticas são de interesse de monitoramento visando a conservação das espécies nativas e a pesca tradicional, como por exemplo: o bagre africano *Clarias gariepinus*, as espécies de piranhas, a vermelha *Pygocentrus nattereri* e a preta *Pygocentrus piraya* e o pacamã *Lophiosilurus alexandri*. Finalmente, a determinação do teor de metais nos peixes é de fundamental relevância para avaliar o seu uso como recurso alimentar pelas populações ribeirinhas.

O estudo avaliando as concentrações de metais em peixes comparando áreas "afetadas" e "não afetadas" pela avalanche de rejeito, pode ajudar a distinguir os efeitos do rejeito dos efeitos de outros poluentes. Os valores registrados nos indivíduos coletados em áreas não afetadas podem indicar uma condição semelhante de contaminação antes do derramamento de rejeito. Caso esta premissa seja verdadeira, esperamos que os animais de áreas "afetadas", que tiveram contato com o rejeito de minério, apresentem concentrações amplificadas pela presença do rejeito.

Esta tese é composta por dois capítulos, o primeiro referente à concentração de metais pesados e arsênio em peixes da bacia do rio Doce e sua relação com as condições físico-químicas da água; no segundo avaliamos bioacumulação e biomagnificação de metais pesados e arsênio nos peixes de água doce de toda a bacia do rio Doce. O primeiro capítulo já foi publicado no periódico Integrated Environmental Assessment and Management (Ferreira et al. 2020, disponível em https://doi: 10.1002/ieam.4289 e conta, até a presente data, com 13 citações no Web of Science e 17 no Google Acadêmico. O segundo capítulo está redigido no formato desta mesma revista, e compreende uma amostragem muito maior, abrangendo a calha e os principais afluentes do alto, médio e baixo rio Doce.

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CAPÍTULO 1

Impacts of the Samarco Tailing Dam Collapse on Metals and Arsenic Concentration in Freshwater Fish Muscle from Doce River, Southeastern Brazil.

Frederico Fernandes Ferreira[1]*; Mariella Bontempo Duca de Freitas[2]; Neucir Szinwelski[3]; Natállia Vicente[1]; Laila Carine Campos Medeiros[4]; Carlos Ernesto Gonçalves Reynaud Schaefer[5]; Jorge Abdala Dergam[2]; Carlos Frankl Sperber[6].

[1] Programa de Pós-Graduação em Ecologia, Universidade Federal de Viçosa, Campus Viçosa, Av. P.H. Rolfs s/n, CEP 36570-900, Viçosa, Minas Gerais, Brazil.

[2] Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

[3] Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, Cascavel, Paraná, Brazil.

[4] Fundação Renova, Belo Horizonte, Minas Gerais, Brazil.

[5] Departamento de Solos, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

[6] Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

*Author to whom correspondence should be addressed: Frederico F. Ferreira (frederico.bio@gmail.com).

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ABSTRACT

In November 2015, Samarco tailings dam in Mariana, Minas Gerais, Brazil, collapsed, releasing 62 million tons of tailings that advanced through 668 km of the Doce River and adjacent floodplain. Although the collapse was the worst environmental disaster in Brazil, little is known about its consequences to aquatic biota. Here we evaluate the effects of the tailings mudflow on metal and As concentration in fish and how concentration correlates with water and fish characteristics. We quantified semi total amounts of Ag, Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in fish muscle tissue using inductively coupled plasma mass spectrometry (ICP-MS) in 255 individuals (34 species) sampled in unaffected and affected areas along the Doce River basin. Arsenic and Hg were higher in fish from affected sites, likely due to turbulent mixing of previously sedimented material by the giant tailings wave. Silver and Zn concentrations were higher in unaffected sites. Arsenic concentration in Geophagus brasiliensis decreased with increasing fish weight. Copper and Zn decreased with

increasing fish weight considering the whole assembly of fish. The tailings mudflow increased water conductivity, and conductivity increased Al concentration in fish, so we expected a larger Al concentration in fishes from affected sites. However, the observed Al concentration in fishes from affected sites was lower than expected by water conductivity. Thus, the tailings mudflow reduced Al uptake or accumulation in fishes. Mercury decreased with increasing water conductivity in both unaffected and affected sites considering all species and in G. brasiliensis alone. Despite the relatively low concentration range of metals and As found in fish, fishes from sites affected by the Fe ore tailings mudflow showed higher As and Hg concentration, compared to fishes from unaffected sites. The higher As and Hg in affected sites require further detailed monitoring to ensure safeguards of human health by fishing activity along the Doce River.
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Keywords: Toxicity, Bioaccumulation, Biodilution, Arsenic, Mercury.

INTRODUCTION

The Samarco dam rupture on 5 November 2015, in Mariana, Minas Gerais, Brazil, was one of the largest ever reported failures of a tailing dam worldwide (Hatje et al. 2017). The dam collapse released more than 62 million tons of Fe ore tailings into the riverbeds and floodplains, reaching the Gualaxo do Norte, Carmo, and Doce rivers, causing the death of 19 people and polluting 668 km of downstream watercourses down to the Atlantic Ocean (Fernandes et al. 2016; dos Santos Vergilio et al. 2020). The Doce River has a long history of land degradation and unplanned water use, with high rates of deforestation followed by erosion, sedimentation, pollution, and eutrophication (Pires et al. 2017). Gold mining, intense in the area since the 17th century up to present days, polluted the riverbeds with toxic elements, such as Hg and As (Hatje et al. 2017). Although anthropogenic disturbances such as agriculture and mining activities have caused long-term habitat degradation, the dam collapse differs from these sources of pollution due to the large amount and speed that the tailings wave traveled, altering the structure of the habitats, such as alluvial soils, river sediments, terraces, floodplains, and river banks (Hatje et al. 2017).

Samarco assured that the tailings waste was composed mainly of inert mineral particles (Escobar 2015). However, several metals and As were detected in the Doce River after the disaster, despite no previous monitoring available for assessing the process and time of contamination (Carvalho et al. 2017; Hatje et al. 2017). This contamination can be potentially harmful for human health and aquatic biota. Here we aimed to evaluate whether the discharge of the Fe ore tailings, produced by the collapse of the Samarco dam, altered the concentration of selected metals and As in fishes from the Doce River basin.

METHODS

Sampling sites and determination of metals and As concentration in fish

We sampled 12 sites within the Doce River basin (Figure 1) from November 2018 to March 2019 (3 y after the Samarco dam rupture). Fish samples and water characteristics were collected in Piranga (sites 01 and 02), Piracicaba (site 03), and Santo Antônio (sites 04, 05, 06, and 07) rivers, areas not reached by the tailings mudflow and considered unaffected sites for the present study (Figure 1). The affected sampling sites included Gualaxo do Norte (site 08), Carmo (site 09), and Doce (sites 10, 11, and 12) rivers (Figure 1). In each sampling site, we set 20 fishing nets of 10m2, comprising 10 different mesh sizes, ranging from 15 to 80mm knot to knot distance. Fish nets were set at sunset and were collected the next morning, after 12 h. Each captured fish was anesthetized with clove oil (Eugenol), identified, weighed (g), measured (mm), sexed, maturation status of each fish's gonad was evaluated macroscopically, and each specimen was photographed. We extracted a sample of approximately 20 g of muscle tissue of each specimen using ceramic knives sterilized with 10% nitric acid. All packaging material and dissection tools, including plastic cutting board, were sterilized before and after using them at each sampling site. All captured fish specimens were packaged in separate plastic bags for each species in the sample, duly labeled with taxonomic classification, sampling site, and collection date, for reference collection, and deposited at the Ichthyological Collection of the João Moojen Zoology Museum of the Federal University of Viçosa (UFV). Fish were fixed in 10% formaldehyde solution for a period of 48 h and then transferred to 70% ethanol solution. Fish sampling, euthanasia, and transport was authorized by the Brazilian Biodiversity Information and Authorization System (SISBIO), Chico Mendes Institute for Biodiversity Conservation (ICMBio), Ministry of Environment (MMA) (SISBIO authorization number 55430-2). One muscle tissue sample of each fish specimen was sent to the Tommasi Ambiental Environment and Food Analysis laboratory, based in Vitória, Espírito Santo, Brazil, for quantitative determination of metals and As. In the laboratory, fish samples (~1.5 g) were crushed and digested in a Microwave Multiwave GO (Anton-Paar, Austria) using a mixture of 65% nitric acid and 30% hydrogen peroxide. The resulting solution was transferred quantitatively to a polypropylene tube and the volume completed with type I water at 10 mL and subsequently analyzed by ICP-MS. Water parameters (pH, O2, and conductivity) were recorded in each site, using an Akso-AK88 (Akso) multiparameter.

Statistical analyses

Response variables were log-transformed to reduce residual asymmetry. All statistical models used normal distribution and were run under R (R Core Team 2019). We used 2 statistical approaches: multivariate and univariate. The multivariate approach included 2- and 3-way multivariate analyses of variance (MANOVAs), both with all fish species combined and with a subset of the collected fish species, including only data on the fish species collected in sufficient abundance in both unaffected and affected sites (*Geophagus brasiliensis*). To deal with the hierarchical structure of our data, in which fish individuals were nested within sites, we included "site" as explanatory factor, analogous to block, in the MANOVAs (Snijders and Bosker 2011). We used the elements as response variables in the MANOVAs, to evaluate the effects on fishes elements profile. When significant effects of the tailings mudflow in the MANOVAs were detected, we investigated which element concentrations were significantly different, comparing unaffected and affected sites using univariate mixed effects models (1-way analyses of variance [ANOVAs] and analyses of covariance [ANCOVAs]), considering each element concentration as the univariate response variable. We present significant results of ANOVAs as mean + standard deviation; significant results for ANCOVAs are presented as scatterplots of log-transformed observed values and lines for values predicted by the minimum adequate models. We used the tailings mudflow as a 2-level explanatory factor, and water parameters (pH, O2, conductivity) and fish characteristics (weight and sex) were covariables, together with the interaction terms between fish characteristics and mudflow. The univariate models were adjusted as generalized linear models with mixed effects (GLMMs), with site as random intercept (Gelman and Hill 2006). Through this approach, the hierarchical structure of our data was fully dealt with (Zuur et al. 2009). Univariate ANOVAs and ANCOVAs were run for all fish species combined, disregarding fish species identity, and then again only for G. brasiliensis. Further details on the statistical analyses are available at Supplemental Data Details on the Statistical Analyses, including data transformations and the equations of the multi- and univariate models, and further references. Indication of which fish species were included in each analysis are available at Supplemental Data Table S1.

RESULTS

We sampled 255 adult fish belonging to 34 species (Table 1). Two hundred and two individuals, belonging to 26 species, were collected in unaffected sites; 53 individuals, belonging to 21 species, were collected in affected sites; 13 species were present in both unaffected and affected sites. The profile of the element concentrations in fish muscle tissue was significantly different between unaffected and affected sites (2-way MANOVA; $p< 2.2 \exp^{-16}$; Supplemental Data Table S2). There was significant interaction of fish species with the effect of the tailings mudflow, considering all species combined (3-way MANOVA; $p= 0.014$; Supplemental Data Table S3), as well as a significant effect of fish species ($p< 2\ \exp^{-16}$) and tailings mudflow ($p< 2\ \exp^{-16}$) affecting the profile of the elements concentration in fish. The results found for the analysis when species were combined agreed entirely with the results that we found considering only the 6 fish species that presented at least 3 individuals in unaffected and affected sites (Supplemental Data Table S4). For 2 of these 6 species (*G. brasiliensis* and *Pachyurus adspersus*), we detected an effect of the tailing mudflow on the profile of the elements concentrations (Supplemental Data Table S5), but only *G. brasiliensis* was collected in sufficient number of individuals at both sites to enable further analyses.

Arsenic and Hg concentrations

The concentration of As and Hg in fish muscle tissue were higher in affected compared to unaffected sites (As: $p= 0.00018$; Hg: $p= 0.016$; Figures 2A and 2B; Supplemental Data Table S6). When we included water and fish characteristics as covariables (see Supplemental Data Table S9 for water parameter values), we detected significant interaction of covariables with the effects of the tailing mudflow for As (fish sex × tailings mudflow: $p= 0.018$; fish weight × tailings mudflow: $p= 3.69\ \exp^{-6}$; Supplemental Data Table S7). In the unaffected sites, As concentration increased with fish weight ($p< 2.2\ \exp^{-16}$; Figure 3A). In affected sites, there was no effect of fish weight on As concentration ($p= 0.35$), but males showed higher As concentration than females ($p= 0.0027$; Figure 3A). For *G. brasiliensis*, however, As decreased with increasing fish weight ($p= 0.0078$; Figure 3D; Supplemental Data Table S8), was higher in affected sites ($p= 8.24\ \exp^{-4}$), and showed no differences between gender ($p= 0.64$).

Mercury concentration in fish decreased with increasing water conductivity ($p= 0.0052$; Figure 4B) and was higher in affected than in unaffected sites (Figure 4B). Male fishes showed lower Hg concentration than females (Figure 4B). For *G. brasiliensis*, Hg concentration also decreased with increasing water conductivity ($p= 0.0015$; Figure 4C) and was higher in affected sites ($p= 8.33\ \exp^{-5}$; Supplemental Data Table S8).

Silver and Zn concentrations

Silver ($p = 0.035$) and Zn ($p = 0.041$) concentrations in fish muscle tissue were lower in affected than in unaffected sites (Figures 2C and 2D; Supplemental Data Table S6). Zinc concentration decreased with increasing fish weight ($p = 0.031$; Supplemental Data Table S7; Figure 3C), but this decrease was less evident in affected sites. When we included water and fish characteristics as covariables to explain Ag concentration, the effects of the Fe tailings mudflow disappeared ($p = 0.81$; Supplemental Data Table S7).

Aluminum and Cu concentrations

Aluminum and Cu concentrations did not differ between unaffected and affected sites (Al: $p = 0.86$; Cu: $p = 0.79$; Supplemental Data Table S6), but when we included water and fish characteristics as covariables, there was a significant effect of the tailings mudflow on Al ($p = 0.017$; Supplemental Data Table S7) and a significant interaction of tailing mudflow with fish weight on Cu concentration ($p = 0.044$; Supplemental Data Table S7). Copper concentrations decreased with increasing fish weight (Figure 3B), but this decrease was less steep in affected sites (Figure 3B). Copper concentration was lower in male fishes compared to females (Figure 3B). Aluminum concentration increased with increasing water conductivity ($p = 0.0033$; Figure 4A), and was lower in affected sites ($p = 0.017$).

Cadmium, Cr, Fe, Mn, Ni, and Pb concentrations

Cadmium, Cr, Fe, Mn, Ni, and Pb did not differ between unaffected and affected sites ($p > 0.14$; Supplemental Data Table S6). Including water parameters and fish characteristics as covariables did not reveal any effect of the tailings mudflow on these metal concentrations ($p > 0.14$; Supplemental Data Table S7).

DISCUSSION

To our knowledge, this is the first evaluation of the effects of the Samarco Fe ore dam collapse on the concentration of metals in tissues of freshwater fish from the affected area. Our results demonstrate that As and Hg concentration in fish was higher in affected than unaffected sites. Segura et al. (2016) verified that the tailings mud did not contain high levels of As or Hg, leading us to discard the hypothesis that the tailing mud itself was the source of the increased levels of these elements in fish tissue from the affected sites. Arsenic has a regional geological affinity with Au-bearing sulfides (Au in arsenopyrite) (Alloway 1990), whereas Hg was used in Au extraction procedures since the 17th century, mainly in the upper Doce River tributaries, such as Carmo and Gualaxo (Schaefer et al. 2015). This shows the historical transference of both elements to the Doce River. The disturbance of the riverbed sediments caused by the passage of the tailings wave provoked mixing and uplifting of old riverbed sediments, exposing otherwise unavailable and stabilized elements (Lambertz and Dergam 2015). The tailing spill caused a substantial increase in suspended sediment loads and Hg increase in the sediments, whereas As increased in suspended particulate matter (Hatje et al. 2017). Our results are a strong evidence that the disturbance of the river bed, through the tailings mudflow, made As and Hg bioavailable in the affected sites, leading to larger As and Hg concentration in the fishes from these sites. Increased As and Hg concentrations in all fish species from affected sites, compared to unaffected sites, are in agreement with the intraspecific results for *G. brasiliensis*, supporting the consistency of these results. The observed increase in As and Hg concentrations in fish from affected sites might be further augmented in the long term, when enhanced erosion caused by heavy rain episodes may lead to remobilization and transport of contaminated particles to the affected sites (Hatje et al. 2017).

Zinc concentration was lower in fishes of affected sites, compared to unaffected sites, which shows that the tailing mudflow reduced the bioavailability of Zn or the tailings mudflow washed the formerly bioavailable Zinc in the affected sites. The tailing mudflow might also have diluted Zn concentration in the water or the interference of the tailings mudflow on water parameters might have reduced Zn uptake by fishes, altering chemical processes of metal elements' availability (Bas¸yigˇ it and Tekin-Özan 2013) and absorption by the fishes (Niyogi and Wood 2004).

Once the increase of Al uptake with water conductivity was taken into account, we unveiled that Al uptake was lower in affected sites (Figure 4A). Thus, although the alterations in water parameters caused by the tailings mudflow (increased water conductivity) should increase Al concentration (as observed in the unaffected sites), there was a further, yet unknown, alteration in affected sites, which reduced Al uptake or accumulation in fishes. This reduction in Al concentration countered also the increased dissolved Al levels in affected sites compared to unaffected sites, detected by Hatje et al. (2017). Thus, our results indicate that there was a physical–chemical or biological alteration, provoked by the tailings mudflow, that countered the Al bioavailability processes related to water conductivity and countered the larger dissolved Al brought by the tailings mud. Further studies on fish physiology and Al bioaccumulation are necessary to unveil these processes.

Relationship of As, Cu, and Zn with fish weight

Our results show evidence of As bioaccumulation with weight in unaffected sites, but not in affected sites, where this element showed higher levels of concentration in fish muscle tissue irrespective of fish weight, showing that in affected sites the bioaccumulation process with fish weight was absent (Figure 3A). This result might be an artifact of the changes in As concentration caused by the tailings mudflow and the short-term contact of fishes with this new environmental condition. If this result is actually an artifact, this pattern should change, once the continuous exposure to As eventually results in its bioaccumulation with fish weight (Kumari et al. 2017). On the other hand, our results might reveal a change in fish uptake or accumulation of As in affected sites. Another important aspect of this result is the chronic As contamination revealed in our study through the bioaccumulation of this element in unaffected sites, which highlights the historical mismanagement of water resources in these areas.

Considering our results for G. brasiliensis, As concentration decreased with increasing body weight in both unaffected and affected sites. This finding suggests that, in this species, the excretion of As overcomes its uptake. Fish have evolved different mechanisms for biotransformation of As to less toxic forms, which are then readily excreted (Bears et al. 2006). Geophagy is frequently related to As exposure in primates (Krishnamani and Mahaney 2000), and it is likely that it plays a role also in this fish species, which is a bottom feeder (Abelha and Goulart 2004). An efficient As excretion would have a high adaptive value for a species that ingests high levels of toxic compounds. Thus, decreased levels of As in fish with higher body weights might be a specific adaptation for these G. brasiliensis populations. Further studies would be necessary to evaluate this hypothesis.

In contrast to As, Cu and Zn concentration decreased with increasing fish weight in both sites, which may be an evidence of some sort of biodilution. Yohannes et al. (2013) verified Cu interspecific biodilution in fish. Biodilution is commonly associated with an interspecific process along the food web, but it may also occur intraspecifically, as when it results from fast growth rates (Pickhardt et al. 2002). For both Cu and Zn, the decrease with fish weight was less steep in affected than unaffected sites, indicating a convergent reduction in the bioaccumulation of these elements in the sites affected by the tailings mudflow. Goethite (FeO(OH)), abundant in Fe ore tailings (Weissenborn et al. 1994), adsorbs Cu (Christophi and Axe 2014). Thus, the tailings mud might have reduced these elements' bioavailability in the affected sites through adsorption by goethite.

CONCLUSIONS

Despite the relatively low concentration range, river waters affected by the Fe ore tailings mudflow, provoked by the Samarco Fe ore dam rupture, showed higher As and Hg concentration in fish muscle tissue. The tailings mudflow also decreased water conductivity and altered As, Al, Cu, and Zn bioaccumulation in fish, showing that environmental assessment of metal and As elements in biota should include water chemistry, weight, and sex of fish as covariables. The higher Hg and As in affected sites requires further detailed monitoring to ensure safeguards of human health by fishing activity along the Doce River. Long-term studies should include testing for synergistic effects, as well as for effects of water chemistry on bioavailability and bioaccumulation, in order to provide a sound and safe understanding of element effects on the biota and human food safety.

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FIGURES

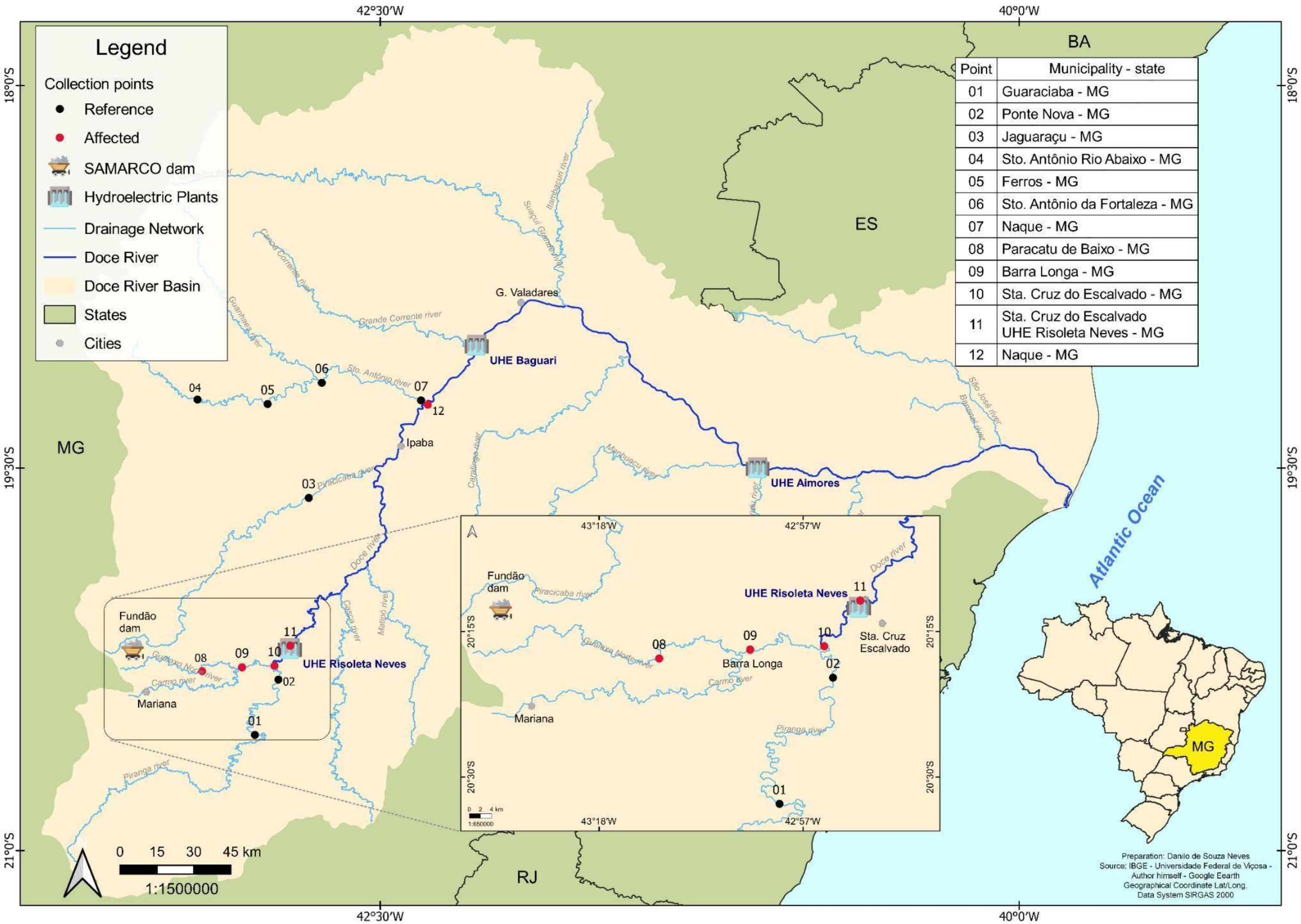

Figure 1. Map of the Doce River basin (in light rose), in Minas Gerais and Espírito Santo states, Brazil. Sampling sites are represented as black (unaffected) and red (affected) circles.

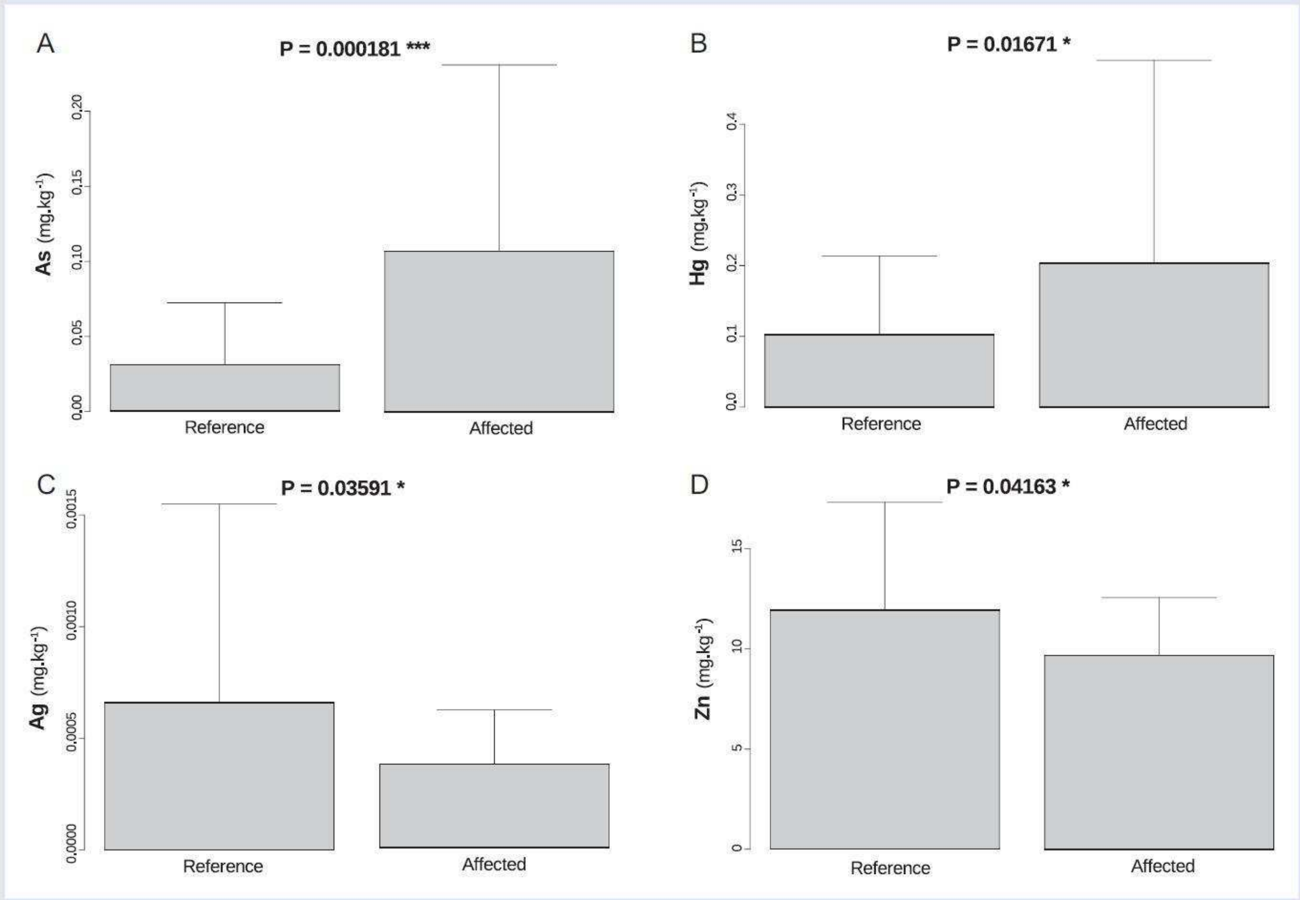

Figure 2 Metals and arsenic concentrations (Means ± SD) in fish muscle tissue (mg.kg−1) of all species combined; (A) arsenic (As), (B) mercury (Hg), (C) silver (Ag), and (D) zinc (Zn) in sites that were unaffected or affected by the iron ore tailings mudflow that resulted from the Samarco dam rupture. P-values refer to mixed effects ANOVAs (GLMMs), with site as random intercept. Only significant results ($P < 0.05$) are presented.

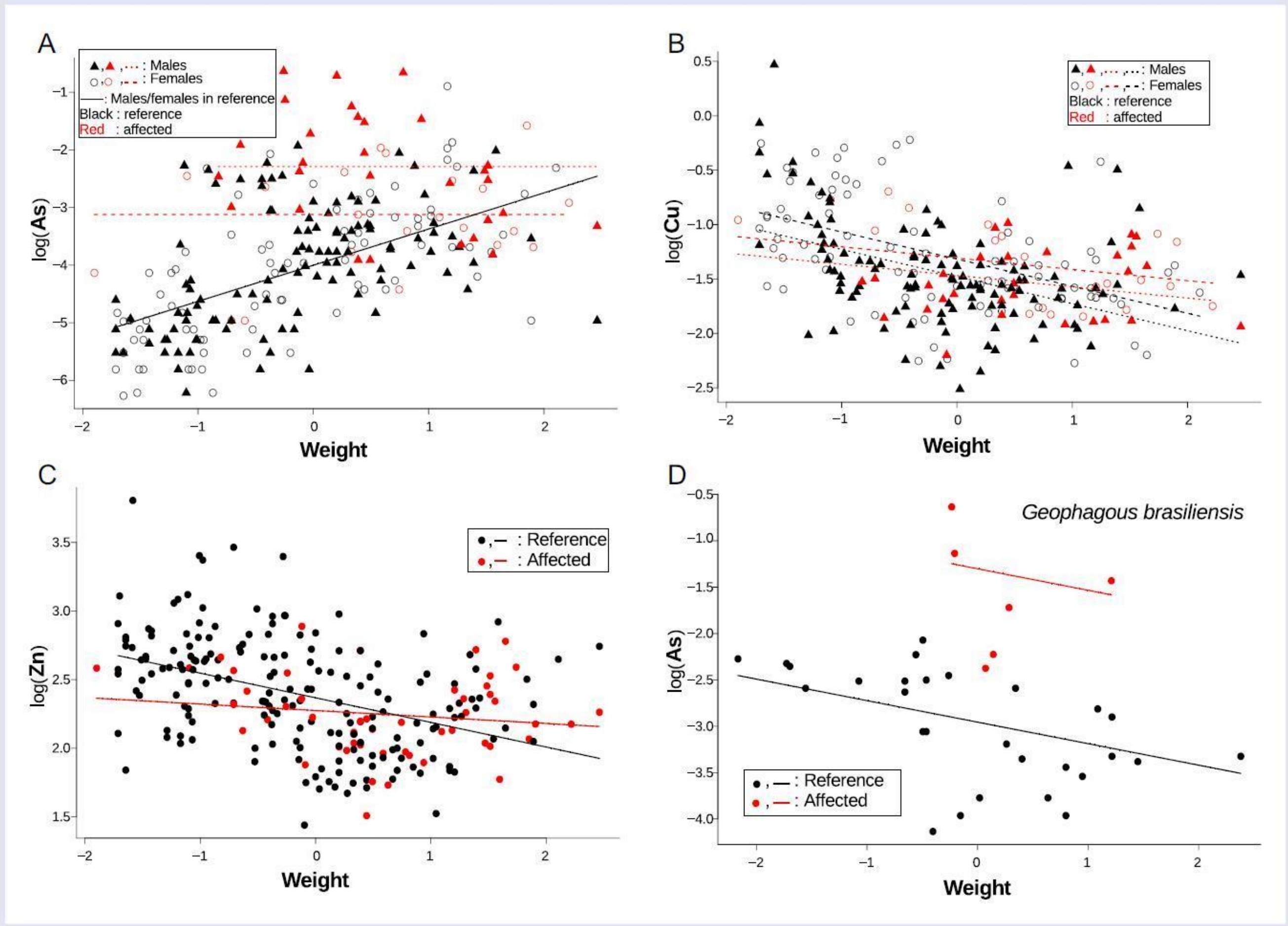

Figure 3. Concentration (log transformed) in fish muscle tissue, in relation to fish weight (centered and scaled), considering the whole fish species assembly, of (A) arsenic (As), (B) copper (Cu) and (C) zinc (Zn); and (D) arsenic (As) within *Geophagus brasiliensis*, comparing fishes from unaffected (black) and from affected (red) sites. Lines refer to predicted values for the minimum adequate mixed effects model (ANCOVA Generalized Linear Mixed Effects Model), with site as random intercept. Lines parallel to the horizontal axis mean that fish weight did not affect the response variable; when there are parallel lines (the red lines in (A), same color lines in (B), red and black lines in (D), this means that there was no interaction of the categorical explanatory variables – sex in (A) and (B), unaffected vs. affected sites in (D), with fish weight, meaning that the effect of weight was the same between fish sexes (A, B) or between unaffected and affected sites (D). Different lines parallel to each other mean significant effect of the categorical variable (sex in (A) and (B), unaffected vs. affected sites in (D)). (A) As increased with fish weight in unaffected sites, but not in affected sites, and As was higher in males than in females in affected sites; (B) Cu decreased with fish weight in both unaffected and affected sites, but this decrease was steeper in unaffected than in affected sites, and Cu was higher in females than males in both affected and unaffected sites; (C) Zn decreased with fish weight in unaffected, but not in affected sites; (D) As in *Geophagus brasiliensis* decreased with fish weight in both unaffected and affected sites and was higher in affected sites than in unaffected sites.

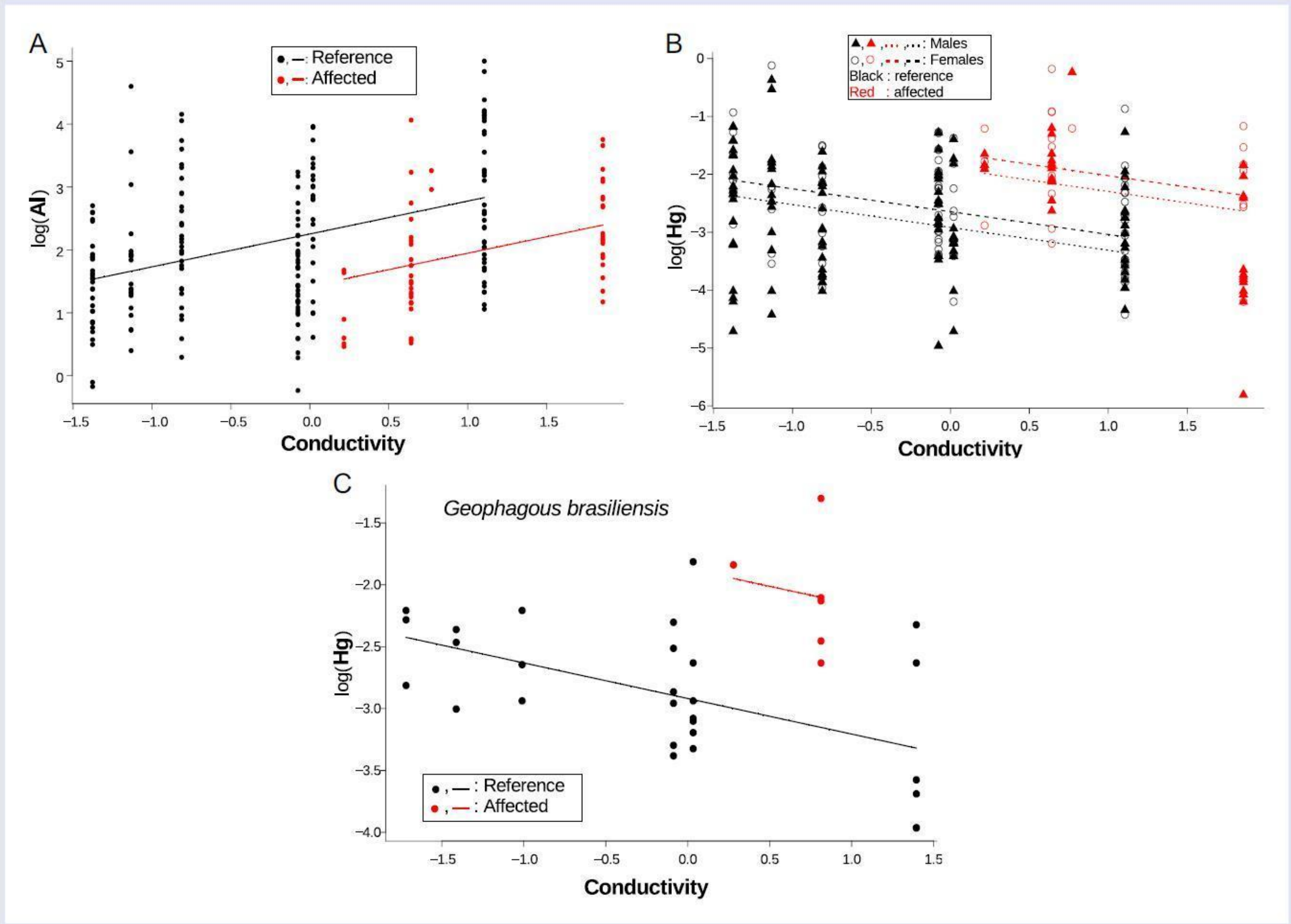

Figure 4. Concentration (log transformed) in fish muscle tissue, in relation to water conductivity, considering the whole fish species assembly, of (A) aluminum (Al) and (B) mercury (Hg); and of (C) mercury (Hg) within *Geophagous brasiliensis*, comparing fishes from unaffected (black) and from affected (red) sites. Lines refer to predicted values for the minimum adequate mixed effects model (ANCOVA Generalized Linear Mixed Effects Model), with site as random intercept. (A) Al increased with water conductivity and was lower in affected than in unaffected sites; (B) Hg decreased with watr conductivity, was higher in affected sites, and was higher in females than in males; (C) Hg in *Geophagous brasiliensis* decreased with water conductivity and was higher in affected than in unaffected sites.

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SUPPLEMENTAL DATA

Supplemental Data Table S1. Number of fish individuals collected per species in affected (n=5) and reference (n=7) sites in the Doce river basin, ordered according to decreasing accumulated abundance, and number of sites where the species occurred. The last five columns point the species included in each of the statistical analyses. The numbers between parentheses refer to the equation of the statistical model mentioned in the Statistical Analyses section of the Methodology.

sp #	Fish species	Affected sites	Reference sites	Total	Number of sites	Two-way MANOVA (eq. 1)	Three-way MANOVA (eq. 2)	Strict three-way MANOVA (eq. 2)	Intraspecific MANOVAs (eq. 2, 3)	GLMMs ANOVAs and ANCOVAs (eq. 4, 5)	Intraspecific GLMM ANCOVA (eq. 6)
1	*Geophagus brasiliensis*	6	30	36	9	x	x	x	x	x	x
2	*Oligosarcus argenteus*	1	27	28	8	x	x		x	x	
3	*Astyanax lacustris*	0	21	21	6	x	x			x	
4	*Hoplias intermedius*	7	10	17	8	x	x	x	x	x	
5	*Hypostomus affinis*	4	13	17	6	x	x	x	x	x	
6	*Pachyurus adspersus*	1	16	17	5	x	x		x	x	
7	*Loricariichthys castaneus*	2	14	16	4	x	x		x	x	
8	*Megaleporinus conirostris*	4	9	13	6	x	x	x		x	
9	*Astyanax sp. 2*	0	10	10	2	x	x			x	
10	*Hypomasticus mormyrops*	0	10	10	2	x	x			x	
11	*Pimelodus maculatus*	4	3	7	3	x	x	x		x	
12	*Oreochromis niloticus*	3	4	7	4	x	x	x		x	
13	*Henochilus wheatlandii*	0	7	7	2	x	x			x	
14	*Hoplias malabaricus*	5	1	6	2	x	x			x	
15	*Rhamdia quelen*	3	2	5	3	x	x			x	
16	*Hypostomus auroguttatus*	0	5	5	4	x	x			x	
17	*Cichla piquiti*	0	4	4	1	x	x			x	
18	*Leporinus copelandii*	0	4	4	3	x	x			x	
19	*Salminus brasiliensis*	3	0	3	1	x	x			x	
20	*Prochilodus vimboides*	2	0	2	1	x	x			x	
21	*Lophiosilurus alexandri*	1	1	2	2	x	x			x	
22	*Trachelyopterus striatulus*	1	1	2	1	x	x			x	
23	*Astyanax fasciatus*	0	2	2	2	x	x			x	
24	*Crenicichla lacustris*	0	2	2	2	x	x			x	
25	*Cyphocharax gilbert*	0	2	2	1	x	x			x	
26	*Delturus carinotus*	0	2	2	2	x	x			x	
27	*Astyanax sp*	1	0	1	1	x	x			x	
28	*Hoplosternum littorale*	1	0	1	1	x	x			x	
29	*Leporinus macrocephalus*	1	0	1	1	x	x			x	
30	*Myloplus asterias*	1	0	1	1	x	x			x	
31	*Prochilodus costatus*	1	0	1	1	x	x			x	
32	*Prochilodus lineatus*	1	0	1	1	x	x			x	
33	*Gymnotus carapo*	0	1	1	1	x	x			x	
34	*Pseudauchenipterus affinis*	0	1	1	1	x	x			x	
	Total	**53**	**202**	**255**	**12**	**34**	**34**	**6**	**6**	**34**	**1**

Supplemental Data Table S2. Effects of the tailings torrent that resulted from the iron ore dam break on the profile of elements' concentration in fish muscle tissue. Results of the two-way MANOVA for all fish species (eq. 1 in the Methodology).

Term	d.f.	Pillai	aprox. F	num. d.f.	den. d.f.	P (>F)	Significance
Tailing torrent	1	0.40698	13.2682	12	232	< 2.2e-16	***
Site (block)	10	1.60912	3.8514	120	2410	< 2.2e-16	***
Residuals	243						

Supplementary Data Table S3. Effects of the tailings torrent that resulted from the iron ore dam break, of the fish species and its interaction, on the profile of elements' concentration in fish muscle tissue. Results of the three-way MANOVA for all fish species (eq. 2 in the Methodology).

Term	d.f.	Pillai	aprox. F	num. d.f.	den. d.f.	P (>F)	Signiificance
Tailing torrent	1	0.6499	29.085	12	188	< 2e-16	***
Fish species	33	4.4018	3.4935	396	2388	< 2e-16	***
Tailing:species	12	0.8639	1.2865	108	2388	0.01426	*
Site (block)	9	1.6109	3.5608	144	1764	< 2e-16	***
Residuals	199						

Supplemental Data Table S4. Effects of the tailings torrent that resulted from the iron ore dam break on the profile of elements' concentration in fish muscle tissue. Results of the two-way MANOVA for the six fish species that presented at least three individuals in each affected and reference sites (n=97 individuals).

Term	d.f.	Pillai	aprox. F	num. d.f.	den. d.f.	P (>F)	Significance
Tailing torrent	1	0.61437	8.7623	12	66	1.07E-09	***
Fish species	5	2.18516	4.5284	60	350	< 2e-16	***
Tailing:species	8	1.02949	1.5125	96	584	0.01256	*
Site (block)	5	2.10091	2.1665	60	350	2.41E-08	***
Residuals	77						

Supplemental Data Table S5. Effects of the tailings torrent that resulted from the iron ore dam break on the profile of elements' concentration in fish muscle tissue, within the six more abundant species. Results of the two or three-way MANOVAs (eqs. 1 or 3 in the Methodology).

Species	Term	d.f.	Pillai	aprox. F	num. d.f.	den. d.f.	P (>F)	Significance
Geophagus brasiliensis								
	Tailings torrent	1	0.842	7.1275	12	16	2.31E-04	***
	Site (block)	7	3.64	1.9859	84	154	1.18E-04	***
	Residuals	27						
Oligosarcus argenteus								
	Tailings torrent	1	0.659	1.4501	12	9	0.29284	
	Site (block)	6	1.607	1.607	72	84	0.01828	*
	Residuals	20						
Hoplias intermedius								
	Tailings torrent	1	0.788	1.2358	12	4	0.4572	
	Residuals	15						
Hypostomus affinis								
	Tailings torrent	1	0.864	2.1232	12	4	0.2437	
	Residuals	15						
Pachyurus adspersus								
	Tailings torrent	1	0.994	57.55	12	4	0.000683	***
	Residuals	15						
Loricariichthys castaneus								
	Tailings torrent	1	0.978	3.7535	12	1	0.3849	
	Site (block)	2	1.913	3.6483	24	4	0.1082	
	Residuals	12						

Supplementary Data Table S6. Mean concentration in fish muscle tissue from affected and from reference sites, and results of the mixed effects one-way ANOVAs (GLMMs, eq. 4 in the Methodology) to evaluate the effect of the tailings torrent that resulted from the iron ore dam brake on the concentration of elements in fish muscle tissue. Site was adjusted as random intercept.

Element	Affected	Reference	LRT	P value	Significance
Ag	5.50E-04	3.74E-04	4.4016	0.03591	*
Al	13.892	11.125	0.0269	0.8698	n.s.
As	0.031	0.107	14.0190	1.81E-04	***
Cd	6.66E-04	6.00E-04	0.5597	0.4544	n.s.
Cr	0.086	0.021	0.2968	0.5859	n.s.
Cu	0.284	0.250	0.0673	0.7953	n.s.
Fe	12.692	15.139	2.1205	0.1453	n.s.
Hg	0.104	0.203	5.7268	0.01671	*
Mn	1.382	1.233	0.0217	0.8828	n.s.
Ni	0.023	0.027	0.5102	0.4751	n.s.
Pb	0.013	0.008	0.0761	0.7827	n.s.
Zn	11.603	9.678	4.1503	0.04163	*

Supplemental Data Table S7. Effects of the tailings torrent that resulted from the iron ore dam break on the concentration of elements in fish muscle tissue, with water parameters and fish characteristics as co-variables. Results of the mixed effects ANCOVAs (GLMMs, eq. 6 in the Methodology). Site was adjusted as random intercept. Significance was evaluated by deletion of non-significant terms. LRT: Likelyhood ration test; P(>Chi): p-value; n.s.: $P \geq 0.05$; *: $P<0.05$; **: $P < 0.01$; ***: $P < 0.001$. (1) When a term was included in a significant interaction, it could not be tested separately (Crawley, 2013).

Element	Terms	d.f.	LRT	P(>Chi)	Significance
Ag	Tailing torrent	1	0.053	0.8179	n.s.
	Fish sex	1	0.651	0.4198	n.s.
	Fish weight	1	5.759	0.0164	*
	Water pH	1	7.429	0.0064	**
	Water O_2	1	7.687	0.0056	***
	Water conductivity	1	1.168	0.2798	n.s.
	Torrent*Sex	1	2.431	0.1189	n.s.
	Torrent*Weight	1	0.824	0.3640	n.s.
Al	Tailing torrent	1	5.639	0.0176	*
	Fish sex	1	0.429	0.5123	n.s.
	Fish weight	1	3.453	0.0631	n.s.
	Water pH	1	0.002	0.9650	n.s.
	Water O_2	1	0.219	0.6396	n.s.
	Water conductivity	1	8.633	0.0033	**
	Torrent*Sex	1	1.869	0.1717	n.s.
	Torrent*Weight	1	1.420	0.2335	n.s.
As	Tailing torrent	1	-	-	(1)
	Fish sex	1	-	-	(1)
	Fish weight	1	-	-	(1)
	Water pH	1	1.703	0.1919	n.s.
	Water O_2	1	0.046	0.8302	n.s.
	Water conductivity	1	3.380	0.0660	n.s.
	Torrent*Sex	1	5.590	0.0181	*
	Torrent*Weight	1	21.418	3.69E-06	***
Cd	Tailing torrent	1	0.321	0.5712	n.s.
	Fish sex	1	0.021	0.8847	n.s.
	Fish weight	1	2.743	0.0977	n.s.
	Water pH	1	2.119	0.1455	n.s.
	Water O_2	1	3.021	0.0822	n.s.
	Water conductivity	1	1.225	0.2685	n.s.
	Torrent*Sex	1	0.035	0.8513	n.s.
	Torrent*Weight	1	0.776	0.3785	n.s.
Cr	Tailing torrent	1	0.044	0.8341	n.s.
	Fish sex	1	0.220	0.6391	n.s.
	Fish weight	1	5.220	0.0223	*
	Water pH	1	0.037	0.8485	n.s.
	Water O_2	1	0.749	0.3869	n.s.
	Water conductivity	1	2.478	0.1155	n.s.
	Torrent*Sex	1	2.734	0.0983	n.s.
	Torrent*Weight	1	0.575	0.4482	n.s.

Supplemental Data Table 7. *Continuation*

Element	Terms	d.f.	LRT	P(>Chi)	Significance
Cu	Tailing torrent	1	-	-	(1)
	Fish sex	1	8.717	0.0032	**
	Fish weight	1	-	-	(1)
	Water pH	1	0.812	0.3676	n.s.
	Water O_2	1	1.261	0.2615	n.s.
	Water conductivity	1	1.725	0.1890	n.s.
	Torrent*Sex	1	0.694	0.4049	n.s.
	Torrent*Weight	1	4.035	0.0446	*
Fe	Tailing torrent	1	0.401	0.5268	n.s.
	Fish sex	1	2.093	0.1480	n.s.
	Fish weight	1	0.013	0.9103	n.s.
	Water pH	1	0.149	0.6991	n.s.
	Water O_2	1	0.500	0.4793	n.s.
	Water conductivity	1	3.439	0.0637	n.s.
	Torrent*Sex	1	2.557	0.1098	n.s.
	Torrent*Weight	1	1.788	0.1812	n.s.
Hg	Tailing torrent	1	10.462	0.0012	**
	Fish sex	1	5.763	0.0164	*
	Fish weight	1	0.357	0.5502	n.s.
	Water pH	1	0.897	0.3437	n.s.
	Water O_2	1	1.722	0.1894	n.s.
	Water conductivity	1	7.784	0.0053	**
	Torrent*Sex	1	1.242	0.2651	n.s.
	Torrent*Weight	1	0.293	0.5884	n.s.
Mn	Tailing torrent	1	0.948	0.3303	n.s.
	Fish sex	1	0.311	0.5769	n.s.
	Fish weight	1	11.532	0.0007	***
	Water pH	1	0.670	0.4129	n.s.
	Water O_2	1	3.345	0.0674	n.s.
	Water conductivity	1	0.376	0.5398	n.s.
	Torrent*Sex	1	1.555	0.2124	n.s.
	Torrent*Weight	1	2.670	0.1023	n.s.
Ni	Tailing torrent	1	0.016	0.9002	n.s.
	Fish sex	1	0.964	0.3261	n.s.
	Fish weight	1	0.397	0.5289	n.s.
	Water pH	1	1.859	0.1727	n.s.
	Water O_2	1	3.012	0.0826	n.s.
	Water conductivity	1	4.064	0.0438	*
	Torrent*Sex	1	0.147	0.7011	n.s.
	Torrent*Weight	1	0.474	0.4914	n.s.

continues

Supplemental Data Table 7. *Continuation*

Element	Terms	d.f.	LRT	P(>Chi)	Significance
Pb	Tailing torrent	1	2.173	0.1405	n.s.
	Fish sex	1	1.802	0.1795	n.s.
	Fish weight	1	17.016	3.71E-05	***
	Water pH	1	1.252	0.2633	n.s.
	Water O_2	1	1.370	0.2418	n.s.
	Water conductivity	1	6.206	0.0127	*
	Torrent*Sex	1	1.276	0.2587	n.s.
	Torrent*Weight	1	2.696	0.1006	n.s.
Zn	Tailing torrent	1	-	-	(1)
	Fish sex	1	0.035	0.8525	n.s.
	Fish weight	1	-	-	(1)
	Water pH	1	0.001	0.9775	n.s.
	Water O_2	1	0.002	0.9672	n.s.
	Water conductivity	1	0.586	0.4441	n.s.
	Torrent*Sex	1	0.910	0.3399	n.s.
	Torrent*Weight	1	4.629	0.0314	*

Supplementary Data Table S8. Effects of the tailings torrent that resulted from the iron ore dam break on the concentration of elements in with water parameters and fish characteristics as co-variables. Results of the mixed effects ANCOVAs (GLMMs, eq. 7 in the Methodology). Site was adjusted as random intercept. Significance was evaluated by deletion of non-significant terms. LRT: Likelyhood ration test; P(>Chi): p-value; n.s.: $P \geq 0.05$; *: $P<0.05$; **: $P < 0.01$; ***: $P < 0.001$. (1) When a term was included in a significant interaction, it could not be tested separately (Crawley, 2013).

Element	**Terms**	**d.f.**	**LRT**	**P(>Chi)**	**Significance**
Ag	Tailing torrent	1	1.803	0.1794	n.s.
	Fish sex	1	0.071	0.7906	n.s.
	Fish weight	1	2.340	0.1261	n.s.
	Water pH	1	12.012	0.0005	***
	Water O_2	1	3.127	0.0770	n.s.
	Water conductivity	1	0.000	0.9947	n.s.
Al	Tailing torrent	1	1.020	0.3124	n.s.
	Fish sex	1	0.224	0.6363	n.s.
	Fish weight	1	0.319	0.5720	n.s.
	Water pH	1	0.226	0.6344	n.s.
	Water O_2	1	0.038	0.8461	n.s.
	Water conductivity	1	2.030	0.1542	n.s.
As	Tailing torrent	1	11.187	8.24E-04	***
	Fish sex	1	0.219	0.6401	n.s.
	Fish weight	1	7.090	0.0078	**
	Water pH	1	0.006	0.9383	n.s.
	Water O_2	1	0.167	0.6829	n.s.
	Water conductivity	1	0.872	0.3505	n.s.
Cd	Tailing torrent	1	2.548	0.1104	n.s.
	Fish sex	1	1.890	0.1692	n.s.
	Fish weight	1	3.155	0.0757	n.s.
	Water pH	1	3.290	0.0697	n.s.
	Water O_2	1	4.020	0.0450	*
	Water conductivity	1	0.459	0.4979	n.s.
Cr	Tailing torrent	1	1.368	0.2422	n.s.
	Fish sex	1	0.036	0.8486	n.s.
	Fish weight	1	0.048	0.8274	n.s.
	Water pH	1	0.171	0.6789	n.s.
	Water O_2	1	0.025	0.8752	n.s.
	Water conductivity	1	0.551	0.4581	n.s.
Cu	Tailing torrent	1	2.249	0.1337	n.s.
	Fish sex	1	0.118	0.7312	n.s.
	Fish weight	1	6.211	0.0127	*
	Water pH	1	2.036	0.1537	n.s.
	Water O_2	1	4.489	0.0341	*
	Water conductivity	1	0.524	0.4693	n.s.

continues

Supplementary Data Table 8. *Continuation*

Element	Terms	d.f.	LRT	P(>Chi)	Significance
Fe	Tailing torrent	1	1.893	0.1689	n.s.
	Fish sex	1	0.033	0.8556	n.s.
	Fish weight	1	0.064	0.8005	n.s.
	Water pH	1	0.047	0.8291	n.s.
	Water O_2	1	0.677	0.4107	n.s.
	Water conductivity	1	2.879	0.0897	n.s.
Hg	Tailing torrent	1	15.482	8.33E-05	***
	Fish sex	1	0.090	0.1134	n.s.
	Fish weight	1	0.987	0.3205	n.s.
	Water pH	1	0.013	0.9084	n.s.
	Water O_2	1	0.150	0.6987	n.s.
	Water conductivity	1	10.021	0.0015	**
Mn	Tailing torrent	1	0.017	0.8957	n.s.
	Fish sex	1	0.749	0.3867	n.s.
	Fish weight	1	7.650	0.0057	***
	Water pH	1	2.860	0.0908	n.s.
	Water O_2	1	0.013	0.9107	n.s.
	Water conductivity	1	0.200	0.6547	n.s.
Ni	Tailing torrent	1	0.034	0.8529	n.s.
	Fish sex	1	2.981	0.0842	n.s.
	Fish weight	1	7.786	0.0053	**
	Water pH	1	0.282	0.5955	n.s.
	Water O_2	1	0.495	0.4817	n.s.
	Water conductivity	1	2.584	0.1080	n.s.
Pb	Tailing torrent	1	0.117	0.7324	n.s.
	Fish sex	1	0.355	0.5514	n.s.
	Fish weight	1	1.643	0.1999	n.s.
	Water pH	1	2.243	0.1342	n.s.
	Water O_2	1	1.409	0.2352	n.s.
	Water conductivity	1	6.756	0.0093	***
Zn	Tailing torrent	1	1.882	0.1701	n.s.
	Fish sex	1	1.222	0.2690	n.s.
	Fish weight	1	4.526	0.0334	*
	Water pH	1	0.799	0.3715	n.s.
	Water O_2	1	0.570	0.4501	n.s.
	Water conductivity	1	0.170	0.6801	n.s.

Supplementary Data Table 9. Effects of the tailings torrent that resulted from the iron ore dam break on the concentration of elements in with water parameters and fish characteristics as co-variables. Results of the mixed effects ANCOVAs (GLMMs, eq. 7 in the Methodology). Site was adjusted as random intercept. Significance was evaluated by deletion of non-significant terms. LRT: Likelyhood ration test; P(>Chi): p-value; n.s.: $P \geq 0.05$; *: $P<0.05$; **: $P < 0.01$; ***: $P < 0.001$. (1) When a term was included in a significant interaction, it could not be tested separately (Crawley, 2013).

Element	Terms	d.f.	LRT	P(>Chi)	Significance
Ag	Tailing torrent	1	1.803	0.1794	n.s.
	Fish sex	1	0.071	0.7906	n.s.
	Fish weight	1	2.340	0.1261	n.s.
	Water pH	1	12.012	0.0005	***
	Water O_2	1	3.127	0.0770	n.s.
	Water conductivity	1	0.000	0.9947	n.s.
Al	Tailing torrent	1	1.020	0.3124	n.s.
	Fish sex	1	0.224	0.6363	n.s.
	Fish weight	1	0.319	0.5720	n.s.
	Water pH	1	0.226	0.6344	n.s.
	Water O_2	1	0.038	0.8461	n.s.
	Water conductivity	1	2.030	0.1542	n.s.
As	Tailing torrent	1	11.187	0.0008	***
	Fish sex	1	0.219	0.6401	n.s.
	Fish weight	1	7.090	0.0078	**
	Water pH	1	0.006	0.9383	n.s.
	Water O_2	1	0.167	0.6829	n.s.
	Water conductivity	1	0.872	0.3505	n.s.
Cd	Tailing torrent	1	2.548	0.1104	n.s.
	Fish sex	1	1.890	0.1692	n.s.
	Fish weight	1	3.155	0.0757	n.s.
	Water pH	1	3.290	0.0697	n.s.
	Water O_2	1	4.020	0.0450	*
	Water conductivity	1	0.459	0.4979	n.s.
Cr	Tailing torrent	1	1.368	0.2422	n.s.
	Fish sex	1	0.036	0.8486	n.s.
	Fish weight	1	0.048	0.8274	n.s.
	Water pH	1	0.171	0.6789	n.s.
	Water O_2	1	0.025	0.8752	n.s.
	Water conductivity	1	0.551	0.4581	n.s.
Cu	Tailing torrent	1	2.249	0.1337	n.s.
	Fish sex	1	0.118	0.7312	n.s.
	Fish weight	1	6.211	0.0127	*
	Water pH	1	2.036	0.1537	n.s.
	Water O_2	1	4.489	0.0341	*
	Water conductivity	1	0.524	0.4693	n.s.

continues

Supplementary Data Table 9. *Continuation*

Element	Terms	d.f.	LRT	P(>Chi)	Significance
Fe	Tailing torrent	1	1.893	0.1689	n.s.
	Fish sex	1	0.033	0.8556	n.s.
	Fish weight	1	0.064	0.8005	n.s.
	Water pH	1	0.047	0.8291	n.s.
	Water O_2	1	0.677	0.4107	n.s.
	Water conductivity	1	2.879	0.0897	n.s.
Hg	Tailing torrent	1	15.482	0.0001	***
	Fish sex	1	0.090	0.1134	n.s.
	Fish weight	1	0.987	0.3205	n.s.
	Water pH	1	0.013	0.9084	n.s.
	Water O_2	1	0.150	0.6987	n.s.
	Water conductivity	1	10.021	0.0015	**
Mn	Tailing torrent	1	0.017	0.8957	n.s.
	Fish sex	1	0.749	0.3867	n.s.
	Fish weight	1	7.650	0.0057	***
	Water pH	1	2.860	0.0908	n.s.
	Water O_2	1	0.013	0.9107	n.s.
	Water conductivity	1	0.200	0.6547	n.s.
Ni	Tailing torrent	1	0.034	0.8529	n.s.
	Fish sex	1	2.981	0.0842	n.s.
	Fish weight	1	7.786	0.0053	**
	Water pH	1	0.282	0.5955	n.s.
	Water O_2	1	0.495	0.4817	n.s.
	Water conductivity	1	2.584	0.1080	n.s.
Pb	Tailing torrent	1	0.117	0.7324	n.s.
	Fish sex	1	0.355	0.5514	n.s.
	Fish weight	1	1.643	0.1999	n.s.
	Water pH	1	2.243	0.1342	n.s.
	Water O_2	1	1.409	0.2352	n.s.
	Water conductivity	1	6.756	0.0093	***
Zn	Tailing torrent	1	1.882	0.1701	n.s.
	Fish sex	1	1.222	0.2690	n.s.
	Fish weight	1	4.526	0.0334	*
	Water pH	1	0.799	0.3715	n.s.
	Water O_2	1	0.570	0.4501	n.s.
	Water conductivity	1	0.170	0.6801	n.s.

CAPÍTULO 2

Bioaccumulation and biomagnification in freshwater fishes from Doce River basin and the influence of ore tailings release by SAMARCO dam collapse in Mariana, Brazil

Frederico Fernandes Ferreira[1]*; Mariella Bontempo Duca de Freitas[2]; Jorge Abdala Dergam[2]; Carlos Frankl Sperber[3]

[1] Programa de Pós-Graduação em Ecologia, Universidade Federal de Viçosa, Campus Viçosa, Av. P.H. Rolfs s/n, CEP 36570-900, Viçosa, Minas Gerais, Brazil.

[2] Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

[3] Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

*Author to whom correspondence should be addressed: Frederico F. Ferreira (frederico.fernandes@ufv.br).

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ABSTRACT

Historical processes of exploitation and intense economic activity turned the Doce River basin into one of the most impacted Brazilian drainages. In 2015, the SAMARCO/BHP-Billiton/Vale dam collapse released 50 tons of ore tailings, aggravating environmental degradation. Here we aimed at evaluating metal concentrations in fishes muscle tissue in areas affected by the dam rupture. We sampled the Doce River basin, along the main channel and main tributaries, classifying sampling sites as “affected” (n=20) and “unaffected” (n=20), the last in tributary rivers that had no contact with the ore tailings (control group). Each sample was composed of 20 fishing nets of $10m^2$, set along a 1 km stretch. In the laboratory, fish samples were crushed, digested and analyzed by ICP-MS. To evaluate if the dam rupture increased metals concentrations, we adjusted each element’s concentration as a univariate response variable. For analyses of the effects of the tailings on bioaccumulation and biomagnification, we adjusted mixed effects linear models in R. Fish bioaccumulation processes were not homogeneous, differing between species and chemical elements. Among the twelve analyzed elements, arsenic was the single element that showed higher concentrations in fishes from affected, compared to unaffected sites. Among the studied affluents, fish from the Gualaxo do Norte River were the only ones with average concentration of mercury higher than the limit established by ANVISA as safe for consumption. The high concentration of metals in the studied region and the physicochemical interaction with the ore tailings seem to be responsible for the differences in the concentrations of metals in fish from affected sites. Ore tailings effects modified the bioaccumulation processes, reinforcing the evidence of physicochemical interaction of the ore tailings with the bioavailability of metals and their accumulation in the aquatic biota. This study is an important step to understanding the chronic impacts of dam failure on aquatic biota and to understand metal accumulation in fishes.

Keywords: Metals, Arsenic, Fish Science, Biodilution, Ecology, Trophic Level, Ecotoxicology.

INTRODUCTION

Metals are chemically defined as elements that conduct electricity in free state, have a metallic luster, are malleable and ductile, form salt cations with oxygen-containing anions such as nitrate and sulfate, and form at least one oxide with a reasonably strong base characteristic (Sawyer et al 2003). Metals are currently released into the environment from both geological/natural (e.g., volcanism and weathering of metal-containing rocks) and anthropogenic (e.g., mining, combustion of fossil fuels, industrial, urban and agricultural activities through metallo-pesticides) sources (Chouvelon et al 2017). The term "heavy metal" refers, *stricto sensu*, to those metals which have specific weights above 5 g/cm^3 (Holleman and Wiberg 1985), but the term has been often used as a group name for metals and semimetals (metalloids) associated with contamination and potential toxicity or ecotoxicity (Duffus 2002). Pollution of water bodies with metals and heavy metals (Ag, As, Cd, Cu, Cr, Hg, Ni, Pb, Zn) is a worldwide problem due to the environmental persistence and toxicity of these elements (Rajaei et al 2012).

Some metals are essential to organisms as they form the basis of biochemical and metabolic interrelationships that influence vital factors necessary for the survival, such as enzymes, antioxidants and vitamins (Soetan et al 2010). However, the concentration of the essential metals can differ among groups of organisms, such as plants, animals and microorganisms, or be essential for a given group but superfluous for another (Ali et al 2019). Fish, for example, perform optimally over a specific and relatively low concentration range of Copper (Cu) and Zinc (Zn), but become deficient or intoxicated at extreme concentrations of these metals (Watanabe et al 1997). In contrast, other metals do not have a known physiological function, but are biologically meaningful for their toxicity and potential risk of accumulation in most organisms, even at low concentrations - these are called non-essential metals, for example, cadmium (Cd), mercury (Hg) and lead (Pb) (Ali et al 2019).

In fish, accumulation of metals includes at least two different absorption routes: (1) aqueous absorption of water-soluble chemicals and (2) dietary absorption by ingestion of contaminated food particles. Both routes, combined, are termed *bioaccumulation* (Streit 1998). *Biomagnification* is known as increased trace metal concentration through at least two trophic levels along a food chain (Barwick et al 2003). Eating habits and microhabitat are important factors influencing accumulation and transfer of metals among species (Kehrig et al 2009). Trophic transfer of metals along the food chain is one of the most important means of exposure to contaminants for predatory animals (Hopkins et al 2005), and influences incorporation, biomagnification and geochemical cycles of metals among aquatic animals (Yang et al 2017). *Biodilution* is a process that also occurs among trophic levels in aquatic environments, that is the opposite to biomagnification, i.e., when the concentration of a pollutant decreases along the food chain (Ali et al 2019).

Freshwater fish have low water intake due to their osmoregulation, thus their gills are the main absorption route for dissolved metals in the water (Wood 2011). Fish gills have a large surface area and are in continuous contact with the external water, with a slim water-blood diffusion distance and active transport pumps designed to acquire nutrient ions from external water (Wood 2011). Metal uptake through the gills may occur through three different routes: (i) metal-specific carriers designed to take up essential metals like Cu and Zn, (ii) ‘‘ionic mimicry’’, i.e. active ionic uptake using pathways designed to take up nutrient metals, as a route of entry (e.g., As mimics phosphate, Pb mimics calcium), and (iii) simple diffusion across the gill’s epithelium, driven by electrochemical gradient from water to blood (Busselberg 1995; Bury et al 2003). Through ionic mimicry, metals may actually reduce the absorption of essential nutrient ions into the gills through competitive inhibition, sometimes resulting in the animal's death from the associated deficiency (e.g. hyponatremia, hypocalcemia) (Paula et al 2021). The same three general mechanisms as for gills also apply to metal uptake via gastrointestinal tract (Bury et al 2003; Wood 2011). Furthermore, metals can additionally bind to amino acids (e.g. L-histidine, L-cysteine) and be absorbed by their specific transporters (Glover and Wood 2008).

Dietary absorption of lipophilic contaminants is a secondary route of exposure in aquatic organisms (Randall et al 1998; Weber e Lanno 2001). Several metals and metalloids have lipophilic characteristics, favoring their binding with lipid molecules and rapid absorption by cells, bioaccumulating in tissues (Kelly et al 2004). Some studies describe increased lipids in organisms exposed to metal and metalloids (Melvin 2019). Fish accumulate temporary energy in the form of fatty acids; the storage of these lipids in the form of triacylglycerols and vitellogenin occurs primarily in the liver, being later remobilized to the muscles (Esteves 2011; Senthamilselvan et al 2016). When ingested by another organism, the fats are absorbed in the intestine, carrying the toxic substance, which then accumulates in the predators' fats (Weber e Lanno 2001). At each level of the food chain there is substantial energy loss, an individual predator will consume many individuals, including all of its lipophilic substances (Kelly et al

2004). Bioaccumulation and biomagnification occur when the rate of excretion is lower than the rate of accumulation and small changes in the environment can increase the metal's concentrations in the environment, leading to bioaccumulation along food chains (Ali e Khan 2018). Although biomagnification in nature is likely to be more limited in occurrence than previously thought, there is good evidence that, at least for organic forms of mercury, biomagnification occurs in nature (Cardwell et al 2013).

Although metals cannot be decomposed, persisting in the environment, they can undergo biotransformation. This would increase or decrease its toxicity (e.g. reduction of Fe^{3+} to Fe^{2+} in the gills and intestines, becoming more bioavailable). Other metals can undergo methylation inside the organism's body, such as Hg and As, which would make them storable, but also increasing its toxicity (Wood 2011). Methylmercury is a more harmful form of mercury that is majorly accumulated inside the body, although some of it may be slowly excreted by organisms (Jernelöv et al 1971; Luo et al 2016).

Organisms, particularly those subject to naturally high levels of metal exposure, have mechanisms to sequester and excrete metals such as the formation of distinct inclusion bodies, and/or binding of metals to heat-stable proteins, metallothionein (Vijver et al 2004). A major concern is associated to higher metal exposures, when the excretion rate fails to avoid harm, or when the exposed organism does not have adaptations for the metal excretion (Rainbow et al 2002).

The Doce River basin

The Doce River basin is located in southeastern Brazil, draining an area of 83,431m^2, of which 86.1% is located in the state of Minas Gerais (MG) and 13.9% in Espírito Santo (ES) (Felipe et al 2016). The Doce River channel begins at the meeting of the Piranga with the Carmo rivers, at the municipality of Rio Doce, MG; its sources are located in the Serra da Mantiqueira, municipality of Ressaquinha, MG, where it travels 853 km, to reach the Atlantic Ocean near the village of Regência, municipality of Linhares, ES (Felippe et al 2016). The anthropic occupation of the Doce River basin has been related to mining since the gold rush in the 18th century, mainly in the Carmo headwaters region (municipalities of Ouro Preto and Mariana, Minas Gerais) (Souza e Reis 2006; Sobreira et al 2014). Gold panning was the only technique for extracting gold during the 18th century and part of the 19th century, until the adoption of mercury for the amalgamation of gold particles (Melamed et al 2002, Ramos et al 2005; Tinôco et al 2010). With the decline of gold exploration in the 19th century, economic stimuli gendered by the industrial revolution and the strong demand for agricultural products, intensifying anthropic occupation of the basin (Espindola et al 2016). The construction of the railroad linking Vitória (ES) to Itabira do Mato Dentro (MG), in 1942, accelerated the occupation of the basin and boosted regional economy, mainly agro-pastoral, iron mining, steel industry and forestry of eucalyptus activities (Espindola et al 2016).

These historical processes of extractivism, high population density and intense economic activity, turned the Doce River basin into one of the most impacted Brazilian drainages by anthropic activities (Vicente et al 2021). Sewage pollution is the main cause of water quality degradation in the basin today; of the 230 cities that are part of the basin, 191 (83%) release their waste in natura (untreated) into the rivers. The parameters that present the largest number of violations of water quality in the Doce River basin are total phosphate, fecal coliforms and total coliforms (PIRH-Doce 2010). In addition, there is contamination by rural sewage, through the use of agricultural inputs such as industrial fertilizers in eucalyptus forestry, that aim to raise phosphorus levels in the soil, but produce large amounts of residuals carried to rivers (Conejo et al 2005). Intensive swine production (creation of pigs), mainly in the upper and middle region of the Doce River basin, are correlated to high levels of zinc (Zn) (Dal Bosco et al 2008; Smanhotto et al 2010; Rocha 2016). High concentrations of metals such as mercury (Hg) and arsenic (As) were reported in sediments in the headwater tributaries of the Doce River basin, Carmo and Gualaxo rivers, historically attributed to mining activities, including those already exhausted (Costa et al 2003; IGAM 2020; dos Reis et al 2020). Widespread deforestation in the Doce River basin caused intense soil erosion, and this process affected water quality both by the leaching of eroded metals from rocks and soil into the aquatic environment, and by siltation, which intensifies flooding in the basin, in addition to the impacts the uncontrolled use of agrochemical pesticides (IGAM 2005). Therefore, the state of degradation of the Doce River basin, prior to the disaster, was already worrying in environmental, social and food security terms. The collapse of the SAMARCO/BHP Billiton/Vale dam aggravated these historical degradation processes, making ecological restoration measures urgent, not to get back to the basin’s state just before the disaster, which was already bad, but to get the basin closer to its original state of conservation, before environmental degradation altogether (Azevedo-Santos et al 2021).

The collapse of the SAMARCO/BHP Billiton/Vale dam released approximately 60 tons of tailings into the Doce River basin (Escobar 2015). This huge amount of iron ore waste has to be dealt with (Hartje et al 2017). In its path, the tailings sludge removed 1,500 ha of vegetation, of which 511.08 ha were Atlantic Forest remnants (Fernandes et al 2016; Gerais 2016). The runoff of the discharged tailings buried villages downstream, killing at least 13 people, reaching 663 km of rivers in the basin, altering the quality of watercourses and causing the immediate death of most aquatic organisms (Escobar 2015). The turbidity caused by the high amount of suspended solids led to the death of aquatic plants, due to the impossibility of photosynthesis, and to the death of fish due to asphyxia, evidenced by the registered 11 tons of dead fish (Fernandes et al 2016; Gerais 2016). The large amount of tailings accumulated in the river bed completely altered the habitat and the composition of the benthic biota (Oliveira et al 2017). The tailings functioned as an "asphalting" of the river bed, covering fish microhabitats, foraging areas, reproduction and fish shelters (Hatje et al 2017). Due to its fine granulometry and low fertility, the tailings constitute a physical barrier that makes growth of plant roots difficult, also affecting the development of aerobic microorganisms, important for plant growth (Lacaz et al 2017). In addition to its direct physical effects, ore tailings runoff resuspended sediments from the deeper parts of the river bed, increasing the concentration of suspended toxic metals in the water, previously trapped in the river's bottom (Guerra et al 2017). Although the tailings sludge composition is mainly Fe and Si oxides, high levels of Al, As, Mn and other elements had been reported in the Doce River water and sediments after the disaster, being gradually mobilized to the biota along the river (Hatje et al 2017; Richard et al 2020). There was a temporal decrease in metal concentration in the sediments from the Doce River along the last five years after the dam rupture, but the estuary has been a significant sink of these sediments, accumulating tailings and metals (Gabriel et al 2021). Additionally, the region where SAMARCO's dam collapsed has a geological composition rich in chemical elements such as arsenic and zinc, acting as a source of these elements, carried into the river bed (dos Reis et al 2020). Thus, the physical and chemical changes caused by the SAMARCO/BHP Billiton/Vale dam's collapse, the long-term contamination and the accumulation of metals, is an increasing threat to the aquatic environment, due to its toxicity and consequent bioaccumulation and biomagnification along food chains (Gopinath et al 2010; Gabriel et al 2021; Reis et al 2020).

In this study, we aimed to evaluate if the mining tailings released by the SAMARCO's dam rupture (i) increased the concentration of heavy metals and arsenic in the fishes' muscular tissue; (ii) affected the bioaccumulation or biodilution of these elements within fish species, and if these differed between fish sex; and (iii) affected the biomagnification of the studied elements.

METHODS

Sampling sites and evaluation of metal and As concentration in fish

Sampling was undertaken from November 2018 to March 2019, three years after the SAMARCO's dam rupture. We sampled 40 sites along the Doce River basin, taking care to distribute these sites equally along the whole Doce River channel and its main tributaries (Figure 1): 20 sites were directly affected by the disruption and discharge of tailings (category "affected") and 20 sites (category "unaffected"), where in tributary rivers that had no direct contact with the ore tailings, as a control group. The control group embraced 11 tributaries of the Doce River and one lagoon: (i) Gualaxo do Norte River (site 01, upstream of the affected areas), (ii) Piranga River (sites 05, 06, 16), (iii) Piracicaba River (site 17), (iv) Santo Antônio River (sites 11, 12, 14, 25), (v) Casca River (site 18, 19), (vi) Manhuaçu River (site 33, 34), (vii) Matipó River (site 24), (viii) Suaçuí-Grande River (site 29, 30), (ix) Caratinga River (site 22), (x) Corrente grande River (site 20), (xi) Guandu River (site 35) and (xii) Juparanã-Mirim Lagoon (site 38). These sites were not reached by the tailings mudflow and thus we considered them as control sites for the present study. The "affected" sampling sites included rivers (i) Gualaxo do Norte (site 03), (ii) Carmo (sites 04, 15) and (iii) the whole Doce River channel, from its beginning, through the confluence of the Carmo with the Piranga Rivers, until the Doce River's flows into the sea, at the Regência District, Linhares, ES (sites 02, 07, 08, 09, 10, 13, 21, 23, 26, 27, 28, 31, 32, 36, 37, 39, 40) (Figure 1).

Each sampling site was composed of an approximate stretch of 1km in length, with 20 fishing nets of $10m^2$, comprising 10 different mesh sizes, ranging from 15 to 80mm knot to knot distance. Fish nets were set at sunset and were collected the next morning, after 12h. Each captured fish was anesthetized with clove oil (Eugenol), identified, weighed (g), measured (mm), sexed, maturation status of each fish's gonad was evaluated macroscopically, and each specimen was photographed. We extracted two samples of approximately 20g of muscle tissue of each specimen using ceramic knives sterilized with 10% nitric acid. All packaging material and dissection tools, including plastic cutting board, were sterilized before and after using them at each sampling site. All captured fish specimens were packaged in separate plastic bags for each species in the sample, duly labeled with taxonomic classification, sampling site, and collection date, and deposited at the Ichthyological Collection of the João Moojen Zoology

Museum of the Federal University of Viçosa (UFV). Fish specimens were fixed in 10% formaldehyde solution in the field, and after 48h they were transferred to 70% ethanol solution. Fish sampling, euthanasia and transport were authorized by the Brazilian Biodiversity Information and Authorization System (SISBIO), Chico Mendes Institute for Biodiversity Conservation (ICMBio), Ministry of Environment (MMA) (SISBIO authorization number 55430-2) and Animal Research and Ethics Committees of UFV (CEUA/UFV authorization number 7982018). One of the muscle tissue samples of each fish specimen was kept frozen at -20ºC, as a duplicate, while the other was sent to the Tommasi Ambiental Environment and Food Analysis laboratory, based in Vitória, ES, Brazil, for quantitative determination of metals and As. In that laboratory, fish samples (~1.5g) were crushed and digested in Microwave Multiwave GO (Anton-Paar, Austria) using a mixture of 65% nitric acid and 30% hydrogen peroxide. The resulting solution was transferred quantitatively to a polypropylene tube and the volume completed with type I water at 10 mL and subsequently analyzed by Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

Statistical analyses

We adjusted the logarithm of each element's concentration in fish muscle as response variable, in separate univariate models for each element, adding to the element's concentration value the minimum observed concentration of the element above zero, when there were fishes with zero concentration values, so as to avoid non-existent log numbers. We adjusted mixed effects linear models (lmer procedure in R), with normal error distribution, with site (n=40) and river identity as independent random intercepts, added to fish species as a third random effect in the analyses of biomagnification, as far as in these analyses there were several fish individuals within each species. By including these random intercepts, the hierarchical, nested, structure of our data was fully dealt with (Zuur et al. 2009). Response variables were log-transformed to reduce residual asymmetry.

To evaluate (i) if the mining tailings affected the concentration of heavy metals and arsenic in the fishes' muscular tissue, we compared sites within the category "affected" against sites in the category "unaffected", by adjusting the category of the sampling site as explanatory factor with two levels, thus running analyses of variance (ANOVAs) for each element. To evaluate (ii) if the mining tailings affected the bioaccumulation or biodilution of the studied elements within fish species, and if these differed between fish sex, we adjusted fish weight, fish sex and category of the site ("affected" versus "unaffected") as explanatory variables, together with all two-level interaction terms, thus running ANCOVAs, since fish sex and site category are two-level factors, while fish weight was adjusted as continuous variable. To evaluate (iii) if the mining tailings affected the biomagnification of the studied elements, we used the trophic level, estimated for each fish species in the World Wide Web electronic publication FishBase (Froese & Pauly 2021), as a continuous explanatory variable. We present significant results of ANOVAs as mean ± standard deviation; significant results for ANCOVAs are presented as scatterplots of observed values and lines for values predicted by the minimum adequate models. We evaluated the significance of explanatory terms by deletion, comparing alternative explanatory models (anova procedure in R). All statistical models used normal distribution and were run under R (R Core Team 2021).

RESULTS

Sampling

We collected 1389 adult fish individuals belonging to 73 species, 19 species exotic or alloctone to the Doce River basin, being 727 individuals (48 species, 14 of them alloctone) in unaffected sites, 662 individuals (46 species, 18 of them alloctone), in affected sites. We used the nine most abundant species that had at least 19 individuals in each affected and unaffected sites, to test (i) the effects of the tailings on heavy metals and As concentration in fish tissue and (ii) bioaccumulation within each of the nine most abundant species, comprehending 797 adult fish individuals (Table 2 and Table 4). These fishes represented 57% of all sampled fish specimens. To evaluate (iii) biomagnification we considered all species with at least three individuals in each affected and unaffected sites (Table 1), comprehending 24 species and 1167 adult fish, 84% of the total abundance of sampled fish (Table 5); we chose to increase the number of fish species in these analyses so as to increase the number of species and specimens within each trophic level. We present the whole statistical results related to bioaccumulation and biomagnification in Table 3, Table 4 and Table 5, and describe these results below.

Metal analyses

Ag — Silver

Silver concentration did not differ between fish from “affected” and “unaffected” sites (Table 2). However, in *Geophagus brasiliensis* and *Hypostomus affinis*, there was an interaction between the effect of ore tailings and fish weight, indicating that the tailings altered the bioaccumulation/biodilution processes in both species (Table 3). In *Geophagus brasiliensis*, silver concentration increased with fish weight (bioaccumulation) in “unaffected” sites, while in “affected” sites this process was reversed (biodilution) (χ^2=6.51; p=0.0107; Figure 2). *Hypostomus affinis* presented silver biodilution in “unaffected” sites and bioaccumulation in “affected” sites (χ^2=13.81, p=0.0002, Figure 3).

We detected no biomagnification of silver, but we detected an interaction between the effect of ore tailings and of trophic level, indicating decrease in silver concentrations along the trophic level in the presence of the tailings (bioreduction) and increase along trophic levels in the absence of the tailings (biomagnification) (χ^2=16.18, p= 5.73 e^{-5}, Figure 4, Table 5).

Al — Aluminum

Only for *Oligosarcus argenteus*, aluminum concentration was lower among fish from "affected" sites compared to fish from "unaffected" sites (χ^2=15.22, p=9.56 e^{-5}, Figure 5-A, Table 2). There was biodilution of aluminum in *O. argenteus* (χ^2=6.93, p=0.0084, Figure 5-B) and also an interaction between the effects of weight and of tailings (χ^2=11.60, p=0.0006, Figure 5-B, Table 3) indicating that the presence of the ore tailings altered *O. argenteus'* biodilution processes. Furthermore, *O. argenteus* female fishes presented higher concentrations of aluminum than male fishes (χ^2=6.21, p=0.0126, Figure 5-C), with an interaction, despite subtle, between the effects of sex and weight (χ^2=8.29, p=0.0039), showing that the bioaccumulation process differed between female and male *O. argenteus* (Table 4). There was bioaccumulation of aluminum in *Oligosarcus acutirostris* in both "affected" and "unaffected" sites (χ^2=5.96, p=0.0195, Figure 6, Table 3). Furthermore, bioaccumulation processes differed between females and males of *Astyanax lacustris* (χ^2=6.70, p=0.0095), with bioaccumulation in males, but not in females, in both "affected" and "unaffected" sites (Figure 7, Table 4). *Pachyurus adspersus* showed interaction between the effects of ore tailings, weight and sex, indicating differences in the bioaccumulation processes between male and female fish from "affected" and "unaffected" sites (χ^2=6.69, p=0.0082, Figure 8, Table 4).

Aluminum concentration did not increase along the trophic levels, discarding any evidence for biomagnification (Table 5).

As — Arsenic

The arsenic concentration was higher among fish from "affected" sites compared to fish from "unaffected" sites in seven of the nine studied species: *Astyanax lacustris* (χ^2=12.17, p=0.0004), *Hoplias intermedius* (χ^2=4.73, p=0.0295), *Loricariichthys castaneus* (χ^2=9.68, p=0.005), *Geophagus brasiliensis* (χ^2=6.70, p=0.0096), *Hypostomus affinis* (χ^2=8.60, p=0.0061), *Oligosarcus acutirostris* (χ^2=17.44, p=2.96e^{-5}) and *Pachyurus adspersus* (χ^2=25.28, p=0.0035) (Figures 9 and 10, Table 2). There was bioaccumulation of arsenic in three of the nine species: *Astyanax lacustris* (χ^2=3.82, p=0.0505), *Hoplias intermedius* (X^2=4.72, p=0.0297) and *Loricariichthys castaneus* (χ^2=3.81, p=0.0488) (Figure 9, Table 3) and there was biodilution of arsenic in two of the nine studied species: *Geophagus brasiliensis* (χ^2=4.81, p=0.0282) and *Pachyurus adspersus* (χ^2=10.30, p=0.0014) (Figure 10, Table 3). There was no relation between arsenic concentration and fish weight in *Hypostomus affinis* (χ^2=2.00, p=0.15) nor in *Oligosarcus acutirostris* (χ^2=1.41, p=0.23) (Table 3).

The concentration of arsenic decreased along trophic levels (bioreduction) (χ^2=11.74, p=0.0006) and there was an interaction between the effect tailings and of trophic level: the effects of tailings were higher at lower trophic levels (χ^2=8.02, p=0.0046, Figure 11) (Table 5).

Cd — Cadmium

Cadmium concentrations were lower among fish from “affected” sites compared to fish from “unaffected” sites in three of the nine studied species: *Hoplias intermedius* (χ^2=6.54, p=0.0472), *Oligosarcus acutirostris* (χ^2=17.44, p=0.0103), and *Pachyurus adspersus* (χ^2=28.98, p=7.30 e^{-8}) (Figure 12, Table 2). There was bioaccumulation of cadmium only in *Hoplias intermedius* (χ^2=4.78, p=0.0287, Figure 12). Furthermore, there was an interaction between the effects of weight and tailings on *Loricariichthys castaneus*, showing that in the presence of tailings the relationship reverses from bioaccumulation in “unaffected” areas to biodilution in “affected” areas (χ^2=6.16, p=0.013, Figure 13, Table 3). In *Hypostomus affinis* there were higher concentrations of cadmium in males than in females both in “affected” and “unaffected” areas (χ^2=3.92, p=0.0477, Table 4).

Cadmium concentration did not increase along trophic levels, thus no evidence for biomagnification was detected (Table 5).

Cr — Chromium

Chromium concentration did not differ between fish from “affected” and “unaffected” sites (Table 2). However, we detected bioaccumulation of chromium in *Loricariichthys castaneus* (χ^2=3.90, p=0.0482, Figure 14, Table 3) in males of both site categories and males of “affected” sites, but not females in “affected”, rendering a significant three-level interaction among the effects of ore tailings, sex and weight (χ^2=6.07, p=0.0136, Figure 15, Table 4). These results indicate differences in the bioaccumulation processes of female fish from “affected” sites.

Chromium concentration did not increase along trophic levels, thus discarding any evidence for biomagnification of this element (Table 5).

Cu — Copper

Copper concentration was lower among fish from “affected” sites compared to fish from “unaffected” sites in two of the nine studied species: *Hypostomus affinis* (χ^2=5.49, p=0.0340) and *Loricariichthys castaneus* (χ^2=4.54, p=0.0329) (Figure 16, Table 2). Furthermore, there was an interaction between the effects of weight and of tailings on *Astyanax lacustris*, showing that in the presence of the tailings the relationship reverses from bioaccumulation in "unaffected" areas to biodilution in "affected" areas (χ^2=6.71, p=0.0121, Figure 17, Table 3). There was biodilution of copper in *Geophagus brasiliensis* (χ^2=10.95, p=0.0009, Figure 18, Table 3). In *Hoplias intermedius* there was an interaction between the effects of tailings, sex and weight (χ^2=3.89, p=0.0483, Figure 19, Table 4), with biodilution in female fishes from "affected" and "unaffected" sites and in male fishes from "affected" sites, but bioaccumulation in male fishes from "unaffected" sites.

Copper concentration did not increase along trophic levels, thus discarding any evidence for biomagnification of copper, but an interaction was detected between the effects of tailings and trophic level, showing increase in copper concentrations with trophic level (biomagnification) in the presence of tailings, contrasting with an decrease in copper concentrations with trophic level (bioreduction) in "unaffected" sites (χ^2=7.09, p= 0.0077, Figure 20, Table 5).

Fe — Iron

Iron concentration did not differ between fish from "affected" and "unaffected" sites (Table 2). However, there was biodilution of iron in *Geophagus brasiliensis* (χ^2=19.74, p=2.11e^{-5}, Figure 21) and *Oligosarcus argenteus* (χ^2=3.93, p=0.0473, Figure 22), and bioaccumulation in *Loricariichthys castaneus* (χ^2=5.88, p=0.0162, Figure 23) (Table 3). Furthermore, in *Pachyurus adspersus* there was an interaction between the effects of tailings, sex and weight (χ^2=5.12, p=0.0136, Figure 24, Table 3), indicating differences and bioaccumulation processes between male and female fish from "affected" and "unaffected" sites.

Iron concentration decreased along trophic levels (bioreduction) (χ^2=4.09, p=0.0430) and there was an interaction between the effects of tailings and trophic level, with steeper bioreduction in "unaffected" than in "affected" sites (χ^2=6.91, p= 0.0089, Figure 25, Table 5).

Hg — Mercury

Only *Hoplias intermedius* presented lower mercury concentrations in fishes from "affected" sites, compared to fishes from "unaffected" sites, (χ^2=194.45, p=2.13e^{-6}, Figure 26, Table 2). There was bioaccumulation of mercury in five, of the nine evaluated fish species (Table3): *Hoplias intermedius* (χ^2=26.61, p=5.88e^{-5}, Figure 26), *Astyanax lacustris* (χ^2=11.21, p=0.0009; Figure 27-A), *Oligosarcus argenteus* (χ^2=55.41, p=0.0367; Figure 27-B), *Loricariichthys castaneus* (χ^2=8.55, p=0.0034; Figure 27-C) and *Pachyurus adspersus* (χ^2=39.10, p=8.45e^{-8}, Figure 27-D). Furthermore, there was an interaction between the effects of ore tailings, fish sex and fish weight in two of the nine evaluated fish species (Table 4): *Oligosarcus acutirostris* (χ^2=4.36, p=0.0367; Figure 28-A) and *Hypostomus affinis* (χ^2=4.64, p=0.0038, Figure 28-B), showing that the bioaccumulation processes differed between male and female fishes, and was also influenced by the ore tailings.

Considering all fish species with more than three individuals in “unaffected” as well as in “affected” sites (Table 1), we detected an overall lower mercury concentration in fishes from “affected”, compared to “unaffected” sites (χ^2=7.80, p=0.0061; Figure 29-A; Table 5). Additionally, we detected biomagnification of mercury, increasing its concentration along trophic levels in both “affected” and “unaffected” sites (χ^2=9.37, p= 0.0021, Figure 29-B, Table 5).

Mn — Manganese

Manganese concentration was lower among fishes from “affected”, compared to “unaffected” sites, in two of the nine studied species (Table 2): *Oligosarcus acutirostris* (χ^2=4.03, p=0.0445; Figure 30-A) and *Pachyurus adspersus* (χ^2=2.63, p=0.0427; Figure 30-C). In *Oligosarcus acutirostris* there was an interaction between the effects of fish weight and ore tailings (χ^2=4.11, p=0.0425, Figure 30-B), indicating that the presence of ore tailings affected the bioaccumulation processes in *O. acutirostris*. There was biodilution of manganese in *Pachyurus adspersus* (χ^2=6.59, p=0.0083, Figure 30-D), *Oligosarcus argenteus* (χ^2=5.08, p=0.0241; Figure 31-A) and *Geophagus brasiliensis* (χ^2=32.61, p=8.91e^{-8}; Figure 31-B).

Manganese concentration did not increase along trophic levels, discarding biomagnification of manganese (Table 5).

Ni — Nickel

Only in one of the nine evaluated fish species, we detected effect of ore tailings on nickel concentration (Table 2): *Hoplias intermedius* had lower nickel values among fishes from “affected”, compared to “unaffected” sites (χ^2=4.85, p=0.0275, Figure 32). Only one of the nine evaluated fish species presented effects of fish weight on nickel concentrations (Table 3): we detected an interaction between the effects of fish weight and ore tailings in *Geophagus brasiliensis* (χ^2=5.80, p=0.0160, Figure 33), showing that in *G. brasiliensis* there was a decrease in concentrations of nickel with fish weight (biodilution) in both “affected” and "unaffected" sites, but the presence of the ore tailings increased biodilution, as evidenced by the steeper adjusted curve for these sites (Figure 33). Furthermore, there was an interaction between the effects of ore tailings, fish sex and fish weight in *Pachyurus adspersus* (χ^2=5.61, p=0.0178, Figure 34, Table 4), indicating differences in the effects of the ore tailings on the biodilution processes in males in from "affected" sites, were biodilution of nickel was steeper (Figure 34).

Concentration of nickel decreased along trophic levels (bioreduction), with no effects of the ore tailings on nickel concentration (χ^2=9.55, p=0.00199, Figure 35, Table 5).

Pb — Lead

Only one of the nine evaluated fish species presented an effect of ore tailings on lead concentrations (Table 2): in *Hypostomus affinis*, lead concentration was lower in "affected" than "unaffected" sites (χ^2=7.06, p=0.0078, Figure 36). We detected biodilution of lead in *Oligosarcus argenteus* (χ^2=7.14, p=0.0075, Figure 37), with no effect of ore tailings (Table 3). We detected an interaction between the effects of fish weight and fish sex in *Geophagus brasiliensis* (χ^2=6.89, p=0.0086; Figure 38-A) and in *Pimelodus maculatus* (χ^2=4.96, p=0.0258, Figure 38-B). In both species, female fishes bioaccumulated lead, while male fishes biodiluted lead (Figure 38). Furthermore, there was an interaction between the efects of ore tailings, fish sex and fish weight in *Loricariichthys castaneus* (χ^2=4.20, p=0.0402, Figure 39, Table 4): while females biodiluted lead in "affected" sites and bioaccumulated it in "unaffected" sites, males presented the opposite pattern, biodiluting in "unaffectd" and bioaccumulating in "affected" sites (Figure 39).

Lead concentration did not increase along trophic levels, discarding biomagnification of lead, but we detected an interaction between the effects of ore tailings and trophic level (Table 5), with a decrease in lead concentrations (bioreduction) in the absence of the ore tailings and apparently no effect of trophic level in the presence of the ore tailings (χ^2=7.48, p= 0.0062, Figure 40, Table 5).

Zn — Zinc

Zinc concentration was higher among fish from affected sites compared to fish from unaffected sites in two of the nine species studied (Table 2): *Hoplias intermedius* (χ^2=3.83, p=0.0455; Figure 41-A) and *Loricariichthys castaneus* (χ^2=11.56, p=0.0008; Figure 41-C) (Figure 41, Table 2). We detected biodilution of zinc in *Hoplias intermedius* (χ^2=10.94, p=0.0010, Figure 41-B), with no interaction with the effects of the ore tailings (Table 3). In *Astyanax lacustris* we detected an interaction between the effects of fish weight and ore tailings (χ^2=4.18, p=0.0408; Table 3; Figure 42), with bioaccumulation in the presence of the ore tailings and biodilution in its absence. Furthermore, we detected an interaction between the effects of ore tailings, fish sex and fish weight in *Pachyurus adspersus* (χ^2=4.95, p=0.0260; Table 4; Figure 43): while females biodiluted zinc in affected sites and bioaccumulated it in unaffected sites, males presented the opposite pattern, biodiluting zinc in unaffectd and bioaccumulating it in affected sites.

Zinc concentration did not vary along trophic levels, dicarding biomagnification of zinc (Table 5).

Table 1. List of fish species with at least three individuals in each "affected" and "unaffected" (control) sites (total $\geq$ 6), number of individuals collected in each site category (Unaffected: sites that had no contact with the iron tailings, n = 20; Affected: sites that had direct contact with the iron tailings, n = 20), food guild category of the species, according to the cited references, trophic level of the fish species, according to FishBase estimation, statistical analysis to which each species was submitted (bioaccumulation and/or biomagnification), example of consumed food, according to the cited references, and the references themselves.

Fish species	Unaffected	Affected	Food guild	Trophic level FishBase	Statistical analyses	Example of consumed food	Reference
Astyanax lacustris	70	72	omnivorous	2.8 ± 0.3	Accumulation Magnification	Aquatic and terrestrial insects, plants and algae.	Vilella et al 2002
Cichla kelberi	5	4	piscivorous/ carnivore	4.4 ± 0.5	Magnification	Fish and invertebrates such as shrimp.	Gomiero et al 2009
Cichla monoculus	8	15	piscivorous/ carnivore	4.2 ± 0.7	Magnification	Fish and invertebrates such as shrimp.	Chellappa et al 2003
Clarias gariepinus	7	9	omnivorous	3.8 ± 0.4	Magnification	Generalists, they feed on a wide variety of items such as invertebrates, fish and plants.	Burgess 1989; Teugels 1986
Crenicichla lepidota	3	9	piscivorous/ insectivorous	3.6 ± 0.5	Magnification	Fish, aquatic insects, arachnids and shrimp.	Gurgel et al 1998
Geophagus brasiliensis	91	28	omnivorous/ bentophagous	2.6 ± 0.3	Accumulation Magnification	Fish, aquatic insects, arachnids, shrimp and algae.	Abelha e Goulart 2004: Malabarba et al 2013
Gymnotus sylvius	4	7	piscivorous/ insectivorous	3.4 ± 0.4	Magnification	Small fish, aquatic insects, beetle larvae and shrimp.	Albert et al 1999
Hoplias intermedius	40	54	piscivorous	4.2 ± 0.7	Accumulation Magnification	Mainly fish and eventually insects.	Oyakawa e Mattox 2009
Hoplias malabaricus	15	24	piscivorous	4.5 ± 0.0	Magnification	Mainly fish and eventually shrimp.	Malabarba et al 2013
Hoplosternum littorale	3	7	omnivorous/	2.7 ± 0.0	Magnification	Microcrustaceans, rotifers, aquatic insect larvae	Malabarba et al 2013; Mol 1995

Table 1. List of fish species with at least three individuals in each "affected" and "unaffected" (control) sites (total $\geq$ 6), number of individuals collected in each site category (Unaffected: sites that had no contact with the iron tailings, n = 20; Affected: sites that had direct contact with the iron tailings, n = 20), food guild category of the species, according to the cited references, trophic level of the fish species, according to FishBase estimation, statistical analysis to which each species was submitted (bioaccumulation and/or biomagnification), example of consumed food, according to the cited references, and the references themselves.

Fish species	Unaffected	Affected	Food guild	Trophic level FishBase	Statistical analyses	Example of consumed food	Reference
			bentophagous			and organic matter.	
Hypostomus affinis	41	45	iliophagous	2.2 ± 0.2	Accumulation Magnification	Mainly debris (highly decomposed organic matter), periphyton in rocks.	Duarte et al 2011; Mazzoni et al 2010
Hypostomus luetkeni	26	12	iliophagous	2.2 ± 0.2	Magnification	Mainly debris (highly decomposed organic matter), periphyton in rocks.	Mazzoni et al 1994; Mazzoni et al 2010
Loricariichthys castaneus	56	19	iliophagous	2.5 ± 0.2	Accumulation Magnification	Mainly debris (highly decomposed organic matter), periphyton in rocks.	Reis e Pereira 2000
Megaleporinus conirostris	27	12	omnivorous	2.6 ± 0.1	Magnification	Tendency to carnivory (mainly insects), possibly molluscs, land plants, fruits and small seeds.	Durães et al 2001; Montenegro et al 2010
Oligosarcus acutirostris	25	28	piscivorous/ insectivorous	4.0 ± 0.7	Accumulation Magnification	Aquatic insects and possibly fish.	Santos et al 2014; da Silva et al 2009
Oligosarcus argenteus	67	24	piscivorous/ insectivorous	3.8 ± 0.7	Accumulation Magnification	Aquatic insects and possibly fish.	Santos et al 2014; da Silva et al 2009
Oreochromis niloticus	7	14	omnivorous/ bentophagous	3.8 ± 0.4	Magnification	Feeds items from the bottom of the river like detritus, algae, sediment and aquatic insect larvae.	Oso et al 2006
Pachyurus adspersus	29	23	insectivorous	3.6 ± 0.4	Accumulation Magnification	Invertebrates aquatic (insect and shrimp larvae).	Vieira et al 2015
Pimelodus maculatus	28	55	omnivorous	2.9 ± 0.3	Accumulation Magnification	Fish, land plants, aquatic and terrestrial insects.	Lolis et al 2018; Ramos et al 2011

Table 1. List of fish species with at least three individuals in each “affected” and “unaffected” (control) sites (total $\geq$ 6), number of individuals collected in each site category (Unaffected: sites that had no contact with the iron tailings, n = 20; Affected: sites that had direct contact with the iron tailings, n = 20), food guild category of the species, according to the cited references, trophic level of the fish species, according to FishBase estimation, statistical analysis to which each species was submitted (bioaccumulation and/or biomagnification), example of consumed food, according to the cited references, and the references themselves.

Fish species	Unaffected	Affected	Food guild	Trophic level FishBase	Statistical analyses	Example of consumed food	Reference
Prochilodus vimboides	12	7	iliophagous	2.1 ± 0.1	Magnification	Mainly debris (highly decomposed organic matter) microcrustaceans, molluscs, insect eggs and larvae.	Castro & Vari 2004; Honji et al 2017
Pseudauchenipterus affinis	14	8	omnivorous	3.4 ± 0.4	Magnification	Land plants and aquatic insects.	Torrente et al 2013
Pygocentrus nattereri	6	44	piscivorous/ carnivore	3.7 ± 0.6	Magnification	Mainly fish, possibly other vertebrates and arthropods.	Murari 2003
Rhamdia quelen	4	10	omnivorous	3.9 ± 0.3	Magnification	Fish, crustaceans, insects, vegetable matter and organic detritus.	Gomes et al 2000
Trachelyopterus striatulus	14	35	piscivorous/ insectivorous	3.5 ± 0.4	Magnification	Mainly aquatic and terrestrial insects and possibly fish.	Vieira et al 2014
Total	**602**	**565**					

Table 2. Effects of the iron ore tailings released by the dam rupture, on the **concentrations of heavy metals and arsenic** in fish species with at least 19 individuals per site category: fish species; number of fish individuals (N: total = unaffected + affected). We indicate the significance (exact p-value); "n.s." indicates that the differences were not significant ($p \geq 0.05$); blue color indicates fish species with lower metal concentrations in affected sites; orange color indicates higher concentrations in affected sites.

Fish species	N	Effect	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Astyanax lacustris	144 (70 + 72)	Ore tailings	n.s.	n.s.	0.0004	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Geophagus brasiliensis	119 (91 + 28)	Ore tailings	n.s.	n.s.	0.0096	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hypostomus affinis	86 (41 + 45)	Ore tailings	n.s.	n.s.	0.0061	n.s.	n.s.	0.0340	n.s.	n.s.	n.s.	n.s.	0.0078	n.s.
Hoplias intermedius	94 (40 + 54)	Ore tailings	n.s.	n.s.	0.0295	0.0472	n.s.	n.s.	n.s.	$2.13e^{-6}$	n.s.	0.0275	n.s.	0.0455
Loricariichthys castaneus	75 (56 + 19)	Ore tailings	n.s.	n.s.	0.005	n.s.	n.s.	0.0329	n.s.	n.s.	n.s.	n.s.	n.s.	0.0008
Oligosarcus acutirostris	53 (25 + 28)	Ore tailings	n.s.	n.s.	$2.96e^{-5}$	0.0103	n.s.	n.s.	n.s.	n.s.	0.0445	n.s.	n.s.	n.s.
Oligosarcus argenteus	91 (67 + 24)	Ore tailings	n.s.	$9.56e^{-5}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pachyurus adspersus	52 (29 + 23)	Ore tailings	n.s.	n.s.	0.0035	$7.30e^{-8}$	n.s.	n.s.	n.s.	n.s.	0.0427	n.s.	n.s.	n.s.
Pimelodus maculatus	83 (28 + 55)	Ore tailings	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Total	**797** (447 unaffected + 350 affected)													

Table 3. Effects of fish weight (**bioaccumulation** or **biodilution**) and its interaction with the iron ore tailings (Ore tailings:Weight), on the concentrations of heavy metals and arsenic in fish species with at least 19 individuals per site category. We indicated the significance (exact p.value); "n.s." indicates that the evaluated effect was not significant ($p \geq 0.05$); blue color indicates fish species with biodilution, orange color indicates bioaccumulation.

Fish species	Effect	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Astyanax lacustris n = 144 (70 + 72)	Weight	n.s.	n.s.	0.0505	n.s.	n.s.	n.s.	n.s.	0.0009	n.s.	n.s.	n.s.	n.s.
	Ore tailings:Weight	n.s.	n.s.	n.s.	n.s.	n.s.	0.0121	n.s.	n.s.	n.s.	n.s.	n.s.	0.0408
Geophagus brasiliensis n = 119 (91 + 28)	Weight	n.s.	n.s.	0.0282	n.s.	n.s.	0.0009	$2.11e^{-5}$	n.s.	$8.91e^{-8}$	n.s.	n.s.	n.s.
	Ore tailings:Weight	0.0107	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0160	n.s.	n.s.
Hypostomus affinis n = 86 (41 + 45)	Weight	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Ore tailings:Weight	0.0002	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Hoplias intermedius n = 94 (40 + 54)	Weight	n.s.	n.s.	0.0297	0.0287	n.s.	n.s.	n.s.	$5.88e^{-05}$	n.s.	n.s.	n.s.	0.0010
	Ore tailings:Weight	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Loricariichthys castaneus n = 75 (56 + 19)	Weight	n.s.	n.s.	0.0488	n.s.	0.0485	n.s.	0.0162	0.0034	n.s.	n.s.	n.s.	n.s.
	Ore tailings:Weight	n.s.	n.s.	n.s.	0.0130	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Oligosarcus acutirostris n = 53 (25 + 28)	Weight	n.s.	0.0195	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Ore tailings:Weight	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0425	n.s.	n.s.	n.s.
Oligosarcus argenteus	Weight	n.s.	0.0084	n.s.	n.s.	n.s.	n.s.	0.0473	$5.09e^{-11}$	0.0241	n.s.	0.0075	n.s.

Table 3. Effects of fish weight (**bioaccumulation** or **biodilution**) and its interaction with the iron ore tailings (Ore tailings:Weight), on the concentrations of heavy metals and arsenic in fish species with at least 19 individuals per site category. We indicated the significance (exact p.value); "n.s." indicates that the evaluated effect was not significant ($p \geq 0.05$); blue color indicates fish species with biodilution, orange color indicates bioaccumulation.

Fish species	Effect	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
n = 91 (67 + 24)	Ore tailings:Weight	n.s.	0.0006	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pachyurus adspersus n = 52 (29 + 23)	Weight	n.s.	n.s.	0.0014	n.s.	n.s.	n.s.	n.s.	$8.45e^{-8}$	0.0083	n.s.	n.s.	n.s.
	Ore tailings:Weight	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pimelodus maculatus n = 83 (28 + 55)	Weight	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Ore tailings:Weight	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 4. Effects of fish gender (female or male), its interaction with the fish weight (Fish sex: Weight) and with the iron ore tailings (a three-level interaction ore tailings:weight:sex) on the concentrations of analyzed elements in fish species with at least 19 individuals per site category. We indicated the significance (exact p.value); "n.s." indicates that the evaluated effect was not significant (p≥0.05).

Fish species	Effect	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Astyanax lacustris	Fish sex : Weight	n.s.	0.0095	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Geophagus brasiliensis	Fish sex : Weight	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0086	n.s.
Hypostomus affinis	Fish sex	n.s.	n.s.	n.s.	0.0477	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	ore tailings:Weight:sex	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0038	n.s.	n.s.	n.s.	n.s.
Hoplias intermedius	ore tailings:Weight:sex	n.s.	n.s.	n.s.	n.s.	n.s.	0.0483	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Loricariichthys castaneus	Fish sex	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0443	n.s.	n.s.	n.s.
	ore tailings:Weight:sex	n.s.	n.s.	n.s.	n.s.	0.0137	n.s.	n.s.	n.s.	n.s.	n.s.	0.0402	n.s.
Oligosarcus acutirostris	ore tailings:Weight:sex	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0367	n.s.	n.s.	n.s.	n.s.
Oligosarcus argenteus	Fish sex	n.s.	0.0126	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Fish sex : Weight	n.s.	0.0039	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pachyurus adspersus	ore tailings:Weight:sex	n.s.	0.0082	n.s.	n.s.	n.s.	n.s.	0.0235	n.s.	n.s.	0.0178	n.s.	0.0260
Pimelodus maculatus	Fish sex : Weight	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0258	n.s.

Table 5. Analyzed element in fish species in sites "affected" (n = 20) or "unaffected" (n = 20) by the Samarco tailings dam rupture, in the Doce River basin, Brazil. For each column we indicated the significance (p.value) showed variation in concentration of metals in fish in relation to trophic level, "n.s." indicates that the differences were not significant; blue color indicates fish species with bioreduction, orange color indicates biomagnification.

Effect	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
ore tailings	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.006176	n.s.	n.s.	n.s.	n.s.
trophic level	n.s.	n.s.	0.0006116	n.s.	n.s.	n.s.	0.043096	0.002198	n.s.	0.001996	n.s.	n.s.
ore tailings: trophic level	5.73e-05	n.s.	0.0046066	n.s.	n.s.	0.007719	0.008981	n.s.	n.s.	n.s.	0.006226	n.s.

Table 6. Mean concentration of each element analyzed in the different tributaries studied and in the main channel (Doce), and the number of fishes sampled. In red values above the limits for human consumption (ANVISA).

Rivers	Fishes	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Caratinga	50	0.000	5.940	0.018	0.001	0.028	0.312	10.204	0.074	0.536	0.023	0.006	11.201
Carmo	32	0.000	5.985	0.111	0.000	0.030	0.324	10.980	0.162	1.525	0.024	0.017	13.689
Casca	51	0.000	8.132	0.012	0.001	0.032	0.269	13.438	0.062	0.543	0.019	0.015	10.526
Corrente	22	0.000	2.925	0.011	0.001	0.029	0.262	10.665	0.161	1.371	0.022	0.179	9.080
Doce	612	0.001	6.901	0.106	0.001	0.017	0.252	9.560	0.129	0.693	0.022	0.013	10.071
Gualaxo	21	0.002	8.149	0.047	0.000	0.021	0.475	26.576	0.941	3.804	0.021	0.008	13.225
Guandu	51	0.000	4.161	0.016	0.001	0.013	0.235	6.752	0.169	0.702	0.012	0.008	10.948
Lagoa juparana	18	0.001	4.956	0.073	0.000	0.007	0.174	5.005	0.260	0.302	0.008	0.008	9.306
Manhuacu	126	0.000	5.100	0.016	0.001	0.013	0.256	7.180	0.149	0.703	0.015	0.013	11.716
Matipo	9	0.000	21.101	0.007	0.001	0.057	0.368	24.409	0.065	2.464	0.060	0.018	13.618
Piracicaba	70	0.001	21.339	0.042	0.001	0.220	0.331	18.005	0.070	1.623	0.043	0.028	11.754
Piranga	158	0.001	9.417	0.029	0.001	0.027	0.236	10.296	0.073	0.791	0.026	0.011	10.994
Santo Antônio	122	0.001	9.519	0.022	0.001	0.016	0.285	10.104	0.113	1.412	0.019	0.006	12.062
Suaçuí	47	0.002	15.114	0.017	0.001	0.029	0.220	13.402	0.066	1.166	0.022	0.060	9.656
Concentração média na Bacia	1398	0.001	9.196	0.038	0.001	0.039	0.286	12.613	0.178	1.260	0.024	0.028	11.275

Tabela 7: Mean concentration of each element analyzed for the species recorded and the number of fish sampled. In red values above the limits for human consumption (ANVISA).

Species	Fishes	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Aequidens tetramerus	1	0.00057	1.003	0.013	0.00034	0.017	0.233	4.831	0.084	0.198	0.016	0.007	15.274
Anchoviella cayennensis	13	0.00472	8.397	1.960	0.00230	0.010	0.679	13.659	0.037	0.558	0.009	0.023	22.934
Astronotus crassipinnis	2	0.00040	13.764	0.048	0.00019	0.012	0.170	10.135	0.056	0.241	0.018	0.020	4.814
Astronotus ocellatus	5	0.00006	1.079	0.034	0.00037	0.005	0.291	5.247	0.041	0.363	0.005	0.004	11.190
Astyanax lacustris	142	0.00097	9.028	0.018	0.00054	0.026	0.526	14.553	0.092	1.889	0.053	0.042	15.468
Caranx latus	1	0.00014	6.859	0.057	0.00022	0.008	0.311	7.144	0.117	0.450	0.005	0.022	7.781
Centropomus parallelus	4	0.00070	4.354	0.129	0.00009	0.004	0.093	3.429	0.077	0.387	0.002	0.002	6.187
Cichla kelberi	9	0.00050	3.514	0.013	0.00033	0.013	0.171	4.549	0.201	0.319	0.011	0.007	10.543
Cichla monoculus	23	0.00024	5.461	0.012	0.00019	0.011	0.241	5.829	0.065	0.484	0.011	0.017	9.607
Clarias gariepinus	16	0.00098	8.595	0.015	0.00032	0.017	0.255	11.457	0.115	0.565	0.017	0.011	9.222
Coptodon rendalli	3	0.00063	1.958	0.016	0.00024	0.008	0.220	5.444	0.025	0.353	0.008	0.002	11.427
Crenicichla lacustris	1	0.00005	0.899	0.014	0.00128	0.008	0.319	3.797	0.078	0.630	0.007	0.000	25.308
Crenicichla lepidota	12	0.00034	9.026	0.025	0.00049	0.014	0.188	6.857	0.073	1.342	0.017	0.010	18.142
Cyphocharax gilbert	8	0.00314	15.407	0.015	0.00078	0.040	0.433	18.479	0.032	1.076	0.029	0.042	13.889

continues

Tabela 7: Mean concentration of each element analyzed for the species recorded and the number of fish sampled. In red values above the limits for human consumption (ANVISA).

Species	Fishes	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Delturus carinotus	22	0.00040	10.668	0.034	0.00072	0.031	0.180	11.532	0.014	1.198	0.026	0.026	4.920
Deuterodon giton	3	0.00014	8.266	0.034	0.00006	0.006	0.333	6.339	0.089	1.728	0.007	0.010	21.413
Deuterodon intermedius	4	0.00004	3.506	0.007	0.00015	0.007	0.338	10.016	0.109	0.833	0.000	0.022	20.612
Deuterodon pedri	2	0.00104	19.104	0.025	0.00051	0.047	0.442	19.521	0.119	2.465	0.049	0.034	25.947
Deuterodon sp	2	0.00023	11.492	0.003	0.00221	0.023	0.434	12.318	0.179	2.018	0.021	0.025	21.206
Eucinostomus argenteus	1	0.00066	5.150	0.082	0.00028	0.006	0.153	4.078	0.146	0.323	0.004	0.009	13.196
Eugerres brasilianus	2	0.00009	1.521	0.375	0.00039	0.007	0.167	2.433	0.096	0.174	0.003	0.000	6.954
Genidens genidens	7	0.00057	10.477	0.294	0.00050	0.012	0.176	12.839	0.203	0.261	0.009	0.012	15.534
Geophagus brasiliensis	119	0.00056	6.847	0.070	0.00046	0.018	0.171	7.558	0.093	1.135	0.017	0.018	13.393
Gymnotus sylvius	11	0.00089	36.388	0.056	0.00051	0.036	0.242	24.744	0.121	1.987	0.023	0.040	9.923
Henochilus wheatlandii	8	0.00045	6.151	0.013	0.00086	0.010	0.251	8.589	0.021	1.728	0.015	0.004	12.319
Hoplias intermedius	94	0.00034	5.254	0.034	0.00030	0.015	0.185	6.083	0.158	0.909	0.013	0.004	9.602
Hoplias malabaricus	39	0.00108	3.217	0.032	0.00034	0.012	0.159	4.481	0.217	0.840	0.010	0.004	7.488
Hoplosternum littorale	10	0.00069	4.552	0.064	0.00079	0.029	0.270	13.320	0.131	0.967	0.015	0.035	4.729

continues

Tabela 7: Mean concentration of each element analyzed for the species recorded and the number of fish sampled. In red values above the limits for human consumption (ANVISA).

Species	**Fishes**	**Ag**	**Al**	**As**	**Cd**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Zn**
Hypomasticus copelandii	18	0.00042	3.452	0.022	0.00054	0.013	0.268	8.611	0.092	0.667	0.017	0.005	12.735
Hypomasticus mormyrops	18	0.00060	4.262	0.010	0.00053	0.020	0.276	7.460	0.115	1.256	0.021	0.005	21.531
Hypomasticus steindachneri	2	0.00027	1.875	0.002	0.00021	0.007	0.218	5.698	0.024	0.419	0.010	0.004	11.183
Hypostomus affinis	86	0.00052	14.087	0.118	0.00039	0.100	0.190	16.943	0.020	0.540	0.034	0.015	6.342
Hypostomus luetkeni	38	0.00052	7.997	0.051	0.00063	0.033	0.208	10.556	0.033	0.553	0.037	0.015	6.086
Lophiosilurus alexandri	12	0.00049	27.164	0.022	0.00022	0.033	0.116	20.382	0.124	1.047	0.021	0.016	8.080
Loricariichthys castaneus	75	0.00063	14.702	0.038	0.00050	0.026	0.158	10.989	0.082	0.555	0.019	0.031	5.957
Lycengraulis grossidens	1	0.00063	13.029	0.058	0.00088	0.021	0.206	7.032	0.386	1.088	0.010	0.011	17.256
Megaleporinus conirostris	39	0.00042	9.782	0.046	0.00063	0.212	0.309	10.183	0.112	1.505	0.029	0.010	11.748
Megaleporinus macrocephalus	3	0.00026	1.306	0.072	0.00019	0.006	0.223	6.266	0.102	0.563	0.006	0.001	8.042
Metynnis lippincottianus	8	0.00133	5.137	0.031	0.00048	0.036	0.280	9.571	0.044	1.003	0.012	0.022	10.363
Moenkhausia vittata	1	0.00017	6.311	0.007	0.00042	0.024	0.145	4.591	0.258	1.816	0.056	0.004	13.417
Mugil curema	5	0.00023	2.300	0.220	0.00049	0.005	0.527	12.498	0.064	0.297	0.014	0.008	7.271
Oligosarcus acutirostris	53	0.00065	4.416	0.012	0.00073	0.019	0.208	6.156	0.237	0.656	0.012	0.009	10.286

continues

Tabela 7: Mean concentration of each element analyzed for the species recorded and the number of fish sampled. In red values above the limits for human consumption (ANVISA).

Species	**Fishes**	**Ag**	**Al**	**As**	**Cd**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Ni**	**Pb**	**Zn**
Oligosarcus argenteus	91	0.00065	7.864	0.015	0.00103	0.017	0.288	9.231	0.217	1.126	0.023	0.015	12.651
Oligosarcus solitarius	4	0.00040	21.953	0.011	0.00078	0.020	0.387	14.053	0.375	1.408	0.013	0.005	12.280
Oreochromis niloticus	21	0.00027	5.690	0.055	0.00051	0.013	0.239	9.570	0.040	0.567	0.016	0.022	10.205
Pachyurus adspersus	52	0.00064	6.344	0.072	0.00039	0.014	0.158	5.900	0.104	0.445	0.012	0.007	8.463
Pimelodella lateristriga	2	0.00009	7.963	0.017	0.00026	0.009	0.165	8.647	0.055	0.448	0.012	0.015	9.856
Pimelodus maculatus	83	0.00065	7.205	0.059	0.00066	0.018	0.206	10.623	0.144	0.447	0.014	0.011	8.221
Pogonopoma wertheimeri	6	0.00013	4.647	0.021	0.00155	0.010	0.407	14.857	0.066	0.405	0.013	0.006	8.784
Prochilodus costatus	10	0.00087	3.331	0.059	0.00092	0.012	0.240	8.806	0.049	0.324	0.012	0.004	14.287
Prochilodus lineatus	8	0.00034	5.012	0.059	0.00070	0.012	0.243	10.076	0.041	0.410	0.016	0.010	9.983
Prochilodus vimboides	19	0.00062	5.765	0.053	0.00031	0.017	0.361	11.103	0.022	0.938	0.022	0.005	9.521
Psalidodon fasciatus	13	0.00048	2.885	0.007	0.00034	0.007	0.320	6.218	0.052	0.584	0.012	0.004	15.916
Psalidodon sp	9	0.00047	5.098	0.012	0.00056	0.007	0.446	9.288	0.178	1.502	0.019	0.007	19.553
Pseudauchenipterus affinis	22	0.00158	10.234	0.017	0.00262	0.036	0.433	17.446	0.361	0.689	0.048	0.029	13.133
Pterygoplichthys pardalis	6	0.00189	3.651	0.042	0.00021	0.010	0.152	11.842	0.026	0.233	0.010	0.015	6.047

continues

Tabela 7: Mean concentration of each element analyzed for the species recorded and the number of fish sampled. In red values above the limits for human consumption (ANVISA).

Species	Fishes	Ag	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Pygocentrus nattereri	50	0.00044	2.517	0.042	0.00036	0.009	0.235	5.281	0.280	0.321	0.009	0.009	8.786
Rhamdia quelen	14	0.00126	10.426	0.056	0.00063	0.026	0.349	26.278	0.811	1.865	0.024	0.014	11.884
Salminus brasiliensis	5	0.00026	10.760	0.022	0.00066	0.024	0.284	12.761	0.792	0.757	0.018	0.002	8.052
Synbranchus marmoratus	2	0.00003	12.101	0.035	0.00146	0.043	0.380	21.993	0.097	1.014	0.050	0.100	30.470
Trachelyopterus striatulus	49	0.00065	9.912	0.021	0.00085	0.027	0.301	14.848	0.104	0.463	0.016	0.012	8.821
Média	1389	0.00065	7.887	0.080	0.00061	0.022	0.271	10.246	0.136	0.842	0.018	0.015	12.233

DISCUSSION

Overall effects of the ore tailings on metals and arsenic concentrations in fish

Among the twelve chemical elements analyzed in fish muscle tissue, As showed the highest concentratios in fishes from sites affected by the ore tailings released by the SAMARCO/BHP Billiton/Vale's dam rupture compared to fish from control sites in seven out of nine analyzed fish species; while Zn showed higher concentrations for two out of nine species (Table 2). As far as these two elements are in low concentrations in the ore tailings, compared to their regional level outside the river (Figueiredo et al. 2020), we interpret the higher concentrations of these elements in fish tissue as resulting from a higher bioavailability of Arsenic and Zinc launched by the ore tailings' physicochemical effects on the river bottom's sediment.

In rivers, finer sediments generally accumulate in low energy environments, which would be predominant in Doce River's tributaries rather than its main channel (Figueiredo et al. 2020), but the SAMARCO's dam rupture launched ore tailings avalanche altered this condition. After the avalanche, the bottom of the Doce River channel presented a different situation, because the tailings buried the former River bottom with a slur mainly composed of anthropogenic, extremely fine, sediments of silt and clay, with lower concentration of organic matter, compared to the unaffected tributaries, resulting in unnatural river conditions (Figueiredo et al. 2020; dos Reis et al. 2020). Adsorption and absorption of contaminants to sediments depends on the relative contents of organic matter and clay per surface area (Streit, 1998). Fine sediments are the main transporting sources of anthropogenic metals carried into the river, due to the high adsorption potential of metals and semimetals (Salomons and Förstner, 2010). The adsorption potential of fine sediments, such as ore tailings, can act as both a sink and a source of metals and semimetals, in storage and remobilization of these elements in the aquatic environment (Castillo et al. 2013). These ore tailings characteristics influence the complexation and dissolution of metals and semimetals between sediments and the water column (dos Reis et al. 2020).

Thus, the richness of the elements in the studied region, allied to the physicochemical interaction with the tailings, seem to be responsible for the differences in the concentrations of

As and Zn in fish muscle tissue. The uptake, storage, and sequestration of metals are a threat to the aquatic environment, due to its toxicity, to these elements not being naturally biodegradable and due to them being bioaccumulative along the food chains (Gopinath et al. 2010). The ore tailings of the Fundão dam are composed mainly of iron and manganese (dos Reis et al. 2020), with very low concentration of other elements, such as Arsenic (82,4mg/kg) and Zinc (39,6mg/kg). Thus, we interpret that the high concentrations of Arsenic and Zinc in fish of affected sites should be attributed to regional geological signature of the sediments that were already at the river bottom, and that increased bioavailability of these elements, rather than coming directly from within the ore tailings themselves.

Arsenic is a metalloid element widely diffused in soil and in aquatic environments as a result of geogenic and anthropic processes (WHO, 1981; ATSDR, 2007). In the soil, it is distributed mainly as arsenides of copper, nickel, and iron, or as arsenic sulfide or oxide (As_2O_3); in the water, arsenic is usually found in the form of arsenate (As^{+5}) or arsenite (As^{+3}) and methylated arsenic compounds occur naturally in the environment as the result of biological activity (WHO, 1981; Hughs, 2002). High concentration of arsenic is lethal to most organisms (Bears et al. 2006), while chronic ingestion of arsenic in low concentrations may be related to enzymatic changes in cells, inhibition of DNA repair and altered DNA methylation, changes related to diseases such as cancer, damage to organs, diabetes and cardiovascular disease (Hughs, 2002). Although most published research has focused on the effects of arsenic on mammal health, fish can be particularly vulnerable to aquatic arsenic as they continuously absorb it through breathing through the gills and ingesting contaminated food (Bears et al. 2006). Substantial increase in arsenic levels in aquatic habitats had been directly related to fish mortality and compromised environmental health (Datta et al. 2007).

Zinc is a vital micronutrient for all organisms due to its vital structural and/or catalytic importance in more than 300 proteins that play important roles in reproduction, development, and immune function (Bury et al. 2003). Zinc deficiency can be a serious dilemma for humans: even moderate deficiency can cause problems including anemia, loss of appetite, immune system defects, impaired development and teratogenesis (Castillo-Duran and Weisstaub, 2003; Walker and Black, 2004). However, in aquatic species, high concentrations of zinc are toxic (Bury et al. 2003). In fish, excessive zinc accumulation can generate toxicity by interfering with calcium homeostasis, being a competitive inhibitor for branchial calcium uptake, sharing a common uptake site in the gills, inducing (Qui et al. 2007). Excessive zinc accumulation has been associated with deleterious effects such as hypocalcemia (Spry and Wood, 1985).

Thus, the higher Arsenic and Zinc concentrations in fish muscle tissue seem to be directly related to two factors: high concentrations of these elements in the region that includes the region of Mariana, MG (see Fig. 2 in dos Reis et al. 2020), and the physicochemical interaction of the ore tailings with these elements, changed their bioavailability for the aquatic biota. Sediments sampled downstream from Fundão indicate that the area is enriched with chemical elements, mainly Arsenium and Zinc (dos Reis et al. 2020). More than that, high concentrations of these elements in the sediment have already been reported in the Doce River before the dam rupture (Costa et al. 2003). Arsenic concentrations in the sediment are related to sulphate quartz-dolomitic veins in the presence of the mineral arsenopyrite abundant in the study region, Mariana, MG (de Vicq et al. 2015). The zinc enrichment of the sediments sampled downstream of the Fundão dam may be due to geological anomalies, since previous studies indicate high measured concentrations of zinc (Rodrigues et al. 2013). In the affected areas of the Gualaxo do Norte and Carmo rivers, seasonal variation in rainfall and soil erosion are the main factors related to variations in the bioavailability of metals to the aquatic environment (Santana et al. 2021).

Our results showed that the other analyzed metals obeyed idiosyncratic rules (Table 2), with specific fish species showing lower concentrations of different metals in the affected sites, according to fish species. While in *Hoplias intermedius*, the concentration of three metals was lower in affected sites, in *Hypostomus affinis, Oligosarcus acutirostris* and *Pachyurus adspersus* the concentration of two metals was lower in affected sites, and in *Loricariichthys castaneus* and *Oligosarcus argenteus*, the concentration of only one metal was lower in affected sites.

Bioaccumulation and biodilution of metal and metalloid in fish muscle tissue

Our results indicated that fish bioaccumulation processes are not homogeneous, differing between fish species and chemical elements (Table 3). This shows that (i) there is variation among fish species in their physiological response to elements (either bioaccumulation or biodilution) – compare the horizontal lines in Table 3: while some fish species show some physiological response to several elements, other species show no response to any of them; as well as (ii) there is variation in the frequency of occurrence of fish physiological responses among elements – compare the vertical columns in Table 3: while some elements have frequent physiological responses in the fishes, other elements have rare fish physiological responses (either bioaccumulation or biodilution).

Two of the fish species distinguished themselves from the others with bioaccumulation of a higher number of chemical elements: *Loricariichthys castaneus* (As, Cr, Fe and Hg) and *Hoplias intermedius* (As, Cd and Hg). *Loricariichthys castaneus* is an iliophagous and benthic species usually found in rivers with a sandy or muddy bottom (Reis and Pereira, 2000), thus feeding on and inhabiting close to the river's bottom sediments. *Hoplias intermedius* is a piscivore/carnivore benthic species, feeding mainly on fish and eventually insects (Oyakawa and Mattox, 2009), but also living close to the river's bottom. The river's bottom has high concentrations of contaminants (Streit, 1998). For fish, proximity with sediments and the water close to them, may result in aqueous uptake of water-borne chemicals through the fishes' gill surface, resulting in their bioaccumulation, which is called bioconcentration (Streit, 1998). Streit (1998) suggests that the indirect effect of feeding on bottom-dwelling aquatic invertebrates might be the most important source of (indirect) absorption of sediment-bound contaminants. Thus, benthic habit, food ecology and physiology of both *Loricariichthys castaneus* and *Hoplias intermedius* are complementary mechanisms that converge to similar bioaccumulation processes.

We detected striking differences in the physiological responses of two fish species of the same genus, *Oligosarcus*: while *O. acutirostris* presented bioaccumulation of Al, *O. argenteus* presented biodilution of Al, Fe and Mn, contrasting to bioaccumulation of Hg. Thus, even phylogenetically closely related species may present differences in ecological needs, metabolism or feeding patterns of fish species, which influence the storage capacity and excretion of chemical elements (Terra et al. 2008, Allen-Gil and Martynov 1995).

Biodilution, i.e., the reduction of contaminants' concentration with fish weight, was already detected for As in *Geophagus brasiliensis* (Ferreira et al., 2020) and for Pb in *Cyprinus carpio* (Fernández-Trujillo et al. 2021). Ferreira et al. (2020) proposed that the physiological process of biodilution could be an effective adaptation to contaminated environments. In this work, we detected biodilution of As, Mn, Cu and Fe in *Geophagus brasiliensis*, with no bioaccumulation of any chemical element in this species. *G. brasiliensis* has direct and severe contact with the riverbed substrate and contaminants, due to being benthic, and is omnivorous, feeding mainly on invertebrate aquatic organisms associated with the substrate (Vieira et al. 2015). Fish have evolved different physiological mechanisms for biotransformation of As, adding methyl groups of thiols in the liver to produce less toxic forms such as arsenobetaine in aquatic organisms, which are then readily excreted (Bears et al. 2006). These physiological mechanisms for metal excretion have been previously studied in several fish species (Kawasaki et al. 1982; Mackay and Fraser 2000; Herman et al. 2021), and might be involved in the biodilution of As in *Geophagus brasiliensis* (Ferreira et al. 2020). Additionally, in fish, there is a process of regulation and redistribution of metals, to accumulate them in tissues other than muscle (Albuquerque et al. 2021). In this work we showed that *Pachyurus adspersus* also showed biodilution of As and Mn, suggesting that this species also has physiological mechanisms of excretion or redistribution of these metals to other fish tissues. *P. adspersus* also has its diet composed of aquatic invertebrates (insects and shrimp larvae) (Vieira et al. 2015), although this species is not directly associated with river sediments, suggesting that the food, rather than the sediments or water, would be the source of their contamination.

We found significant bioaccumulation of Hg in five of the nine analyzed species (*Astyanax lacustris, Hoplias intermedius, Loricariichthys castaneus, Oligosarcus argenteus* and *Pachyurus adspersus*) and none of the species presented Hg biodilution. The bioaccumulation in so many fish species confirms the great bioaccumulative potential of this metal in fish (Barbosa et al. 2003; Fernández-Trujillo et al. 2021). Among the fish species that presented bioaccumulation of Hg, *Astyanax lacustris* had the lowest bioaccumulation slope (see Figure 27A). This is an expected result, based on the reasoning that *A. lacustris* is a small omnivore species, contrasting to the other species that bioaccumulate Hg in our study. *H. intermedius*, *L. castaneus*, *O. argenteus* and *P. adspersus*, that are large-sized, piscivorous and have long life cycles, thus longer exposure time, leading to higher levels of Hg than fish having short life cycles and smaller adult sizes (Beltran-Pedreros et al. 2011). This result follows a pattern already described in other studies for fish from the Rio Negro (Barbosa et al. 2003) and for marine fish (Payne and Taylor, 2010), with dietary habits and trophic level determining the amount of bioaccumulation.

Mercury is one of the most widespread and toxic trace elements in the biosphere, can cause neuromotor disturbances and neuropathies, normally related to gold extraction, mainly in the state of Minas Gerais, but may also be related to deforestation of Hg-rich soils for agriculture and leaching to the aquatic environment (Cursino et al. 1999; Clarkson et al. 2003; Telmer et al. 2006; Cesar et al. 2011). The organic form of mercury, methylmercury (MeHg), is of greatest concern because it accounts for the majority of total mercury in fish muscle tissue, being the consumption of contaminated fish the main form of human exposure to mercury (Mergler et al. 2007; Payne and Taylor, 2010). Riverine and indigenous populations or those that live near oceans, major lakes or hydroelectric dams, have high levels of fish consumption and often depend on local catch, with fish an integral part of their cultural traditions, leading them to be more exposed to MeHg contamination (Barbosa et al. 2003; Mahaffey et al. 2004; Mergler et al. 2007; Custodio et al. 2020).

The World Health Organization (WHO) established a provisional tolerable weekly human intake limit of 0.24 mg of total mercury (4 µg/kg of body weight), of which no more than 0.1 mg (1.6 µg/kg of body weight) must be in the form of methylmercury, based mainly on high toxicity of methylmercury and on the relationship between mercury intake in fish associated with clinical development of disease (WHO/FAO, 2011; WHO/FAO, 2016). The legally established concentration limit for human consumption in Brazil is 0.5 mg of total mercury/kg of wet weight of food (ANVISA, 1998) which is based on WHO's mercury ingestion limit for adults, with an average body weight of 63 kg and an assumed food consumption rate of 60 g/day. However, some riverside populations, such as those in the Amazon, consume between 340g and 800 g/day of fish, in periods of abundance, being fish their main source of protein, with fish consumption varying according to cultural practices and seasonal availability (Passos and Mergler 2008). In this case, the limiting value of mercury consumption in fish should be 0.009 mg/kg to 0.02 mg/kg, much lower than the levels indicated as acceptable by ANVISA. For the riverine and indigenous populations, such as those inhabiting the Doce River basin, the daily mercury consumption is particularly difficult to estimate due to the diversity of consumed fish species and environmental heterogeneity, as well as seasonal changes in the availability of some fish species (Beltran-Pedreros et al. 2011; WHO 2008; Junior et al. 2018).

Among the affluents studied, fish from the Gualaxo do Norte River (Mean = 0.94 mg of total mercury/kg; n=21 fish specimens) were the only ones with higher average concentration of mercury than that established by ANVISA for human consumption (0.5 mg of total mercury / kg; ANVISA, 1998) (Tabela 6). The specificity of this affluent explains the higher concentration of Hg in fish of affected sites, attributed by Ferreira et al. (2020) to the presence of the ore tailings. In their study, they considered as affected sites exclusively localities in the Gualaxo do Norte River, compared to fishes from the Santo Antônio river as reference sites. Thus, we attribute Fernandes et al. (2020) results as revealing rather spatial heterogeneity in the history of gold mining among rivers, particularly high in the Gualaxo do Norte River, than to effects of the ore tailings themselves. In the present study, we considered 11 affluents of the Doce River as unaffected reference sites, and also added a reference site in the Gualaxo do

Norte River; thus, we were able to control for river identity as random effects. Thus, our present results in relation to Hg concentration in fish are more robust and are complementary, but not contradictory, to Fereira et al. (2020).

In the present study, we detected high concentrations of mercury in fish from both affected and unaffected sites, indicating that it is not related to the dam failure, but rather to the historical process of gold extraction in the region of Mariana, MG (Souza e Reis 2006; Sobreira et al 2014). The highest mercury values were recorded in the omnivorous catfish *Rhamdia quelen* (5.11 mgHg/kg) and the piscivorous wolf fish *Hoplias intermedius* (3.05 mgHg/kg), both from site 1 (Figure 1), not affected by tailings (municipality of Mariana, in the Gualaxo do Norte River). Among the fish studied, *Rhamdia quelen* (Mean = 0.811 mg of total mercury/kg; n=14 specimens) and the piscivorous *Salminus brasiliensis* (Mean = 0.792 mg of total mercury/kg; n=5 specimens) were the only ones with higher average concentration of mercury than that established by ANVISA for human consumption (0.5 mg of total mercury / kg; ANVISA, 1998) (Tabela 7). Both these fish species are predators: *Rhamdia quelen* is an omnivorous catfish species with a feeding preference for fish, crustaceans and insects; *Hoplias intermedius* and *Salminus brasiliensis* are carnivorous species with feeding preference for fish (Gomes et al. 2000; Carvalho et al. 2002). In freshwater fish, Hg concentrations are usually higher in piscivorous fish (Depew et al 2013; Johnson et al 2015). The high Hg values in fish from this tributary (Gualaxo do Norte River) are a major concern, that will be the focus of future studies, due to the high Hg values regardless of trophic level nor feeding preference of the species, and in both sites affected and unaffected by the ore tailings (Tabela 6). As well as piscivorous species (Tabela 7).

Intra and interspecific differences in metal accumulation in fish

Six of nine evaluated fish species showed significant interaction of the effects of the tailings with weight (Table 3), amplifying, reducing or changing the bioaccumulation process in the presence of ore tailings in at least one of the twelve analyzed elements, reinforcing the evidence of physicochemical interaction of the tailings with the bioavailability of metals and their accumulation in the aquatic biota. *Hypostomus affinis* from affected sites presented bioaccumulation of silver, reversed in non-affected sites (Figure 3). Contrastingly, *Geophagus brasiliensis* has decreasing silver concentration in relation to weight in affected sites (biodilution) and increasing concentration (bioaccumulation) in fish from non-affected sites

(Figure 2). *Astyanax lacustris* also showed biodilution for Cu and Zn in affected sites, contrasting to bioaccumulation in unaffected sites (Figures 17 and 42), similarly to the result for Cd in *Loricariichthys castaneus* (Figure 13), Mn in *Oligosarcus acutirostris* (Figure 30) and Ni in *Geophagus brasiliensis* (Figure 33), all of them with concentrations decreasing (biodilution) in affected sites and increasing (bioaccumulation) in fish from non-affected sites. This interaction effects differed among species and among elements, with no repeating pattern, but evidencing that the consequences of the ore tailings affected fish physiologies. This indicates that anthropogenic sediments, such as ore tailings, can interact with metals by altering the bioavailability of metals in aquatic biota (Yang et al. 2017), varying among fish species and element identity.

An alternative interpretation for the patterns that we called "biodilution" would be that they would reveal rather historical than biological processes: larger concentrations in smaller fish would be rather related to the recent increase in the bioavailability of metals in sites with ore tailings, affecting juvenile (thus smaller) fish, that were in direct contact with ore tailings during its development, unlike larger fish that may have recolonized the main channel from unaffected tributaries by ore tailings. Thus, the larger, heavier fishes would be colonizing individuals, which had not had previous direct contact with the ore tailings. This would mean that the observed intraspecific variations in the concentration of metals could be driven by the fish ontogenetics, which can present a higher rate of absorption in initial phase of life, from larvae to juveniles, with a stable plateau in the post-adult phase (Guo et al. 2016). To evaluate this hypothesis, molecular studies had to unveil differences in intraspecific colonization history related to fish weight.

In five fish species there was an interaction between the effects of the ore tailings, fish sex and fish weight (Table 4), indicating differences in bioaccumulation processes between male and female fish from affected and unaffected sites in *Hypostomus affinis* (Hg), *Oligosarcus acutirostris* (Hg), *Hoplias intermedius* (Cu), *Loricariichthys castaneus* (Cr and Pb) and in *Pachyurus adspersus* (Al, Fe Ni and Zn). The bioaccumulation of metals in fishes might be influenced by the ore tailings, but our results also indicate the interaction of other factors such as fish sex, diet, age, size, weight, fat accumulation, metabolism, metal bioavailability and amount of exposure to this metal (Beltran-Pedreros et al. 2011; Qui et al. 2007; Grosell et al. 2007).

Biomagnification and bioreduction of metal and metalloid in fish muscle tissue

Only Hg presented pure effects of the ore tailings (Table 5), with lower levels in affected sites (Figure 29), besides evidence of biomagnification in both affected and unaffected sites (Table 5). The ore tailings reduced the bioavailability of Hg, despite maintenance of the similar biomagnification slopes (Figure 29), in agreement with previous studies indicating that the ore tailings did not increase genotoxicity potential in the waters of the Doce river (Gomes et al. 2018).

Irrespective of the ore tailings effects, high mercury concentrations in fish in the Doce River basin are probably related to historical processes of gold extraction and liberation of this metal through overall mining, widely distributed in the state of Minas Gerais, which covers a large part of the Doce River basin. In the second largest tragedy involving mining dams in Brazil, the Brumadinho ore tailings' dam collapse of January 2019, a similar result was reported: biomagnification of mercury in fish was not attributed to the tailings dam rupture, since the concentration of Hg in fish was at the same level as before the disaster (Parente et al. 2021). Additionally, in the Brumadinho ore tailings' dam collapse, the concentration of several metals in the sediments was lower after than before the disaster (Vergilio et al 2020).

Biomagnification of mercury was expected, given its already widely described potential for accumulation in its organic form in aquatic biota (Barbosa et al. 2003; Passos and Mergler 2008; Payne and Taylor 2010; Beltran-Pedreros et al. 2011; Cesar et al. 2011; García-Medina et al. 2017; Custodio et al. 2020; Parente et al. 2021), and given our bioaccumulation results detected for Hg in most of the studied fish species.

The metals Fe, Ni and the metalloid As had their concentrations decreased along trophic levels (Figures 25, 35, 11), showing that the concentration of these elements diminishes along trophic levels, which we call bioreduction, the opposite of that expected for biomagnification. Previous studies are consistent, as far as they indicate that, in fish, Fe and Mn accumulate significantly in the lower part of the trophic chain and that no biomagnification occurs (Herman et al. 2021). Lower trophic level fish feed mainly on detritus (highly decomposed organic matter), benthic invertebrates and rock periphyton, foods highly influenced by the availability of metals in the river bottom sediment (Vieira et al. 2015; Wang 2002). Phytoplankton, zooplankton and bacteria play important roles in arsenic and iron speciation, distribution and cycling in aquatic systems, absorbing, accumulating and transforming inorganic forms of arsenic when exposed through their diet, water, soil or suspended particles (Fernández-Trujillo et al. 2021). This suggests that fish in direct contact with the sediment through their food may be more susceptible to the absorption of these metals and metalloids (Łuczyńska et al 2018). Our results indicate that, for Fe, Ni and As in fish, bioaccumulation processes can have opposite effects from biomagnification processes, with intraspecific increase (bioaccumulation) of metals and metalloids, but interspecific decrease (bioreduction) along the food chain.

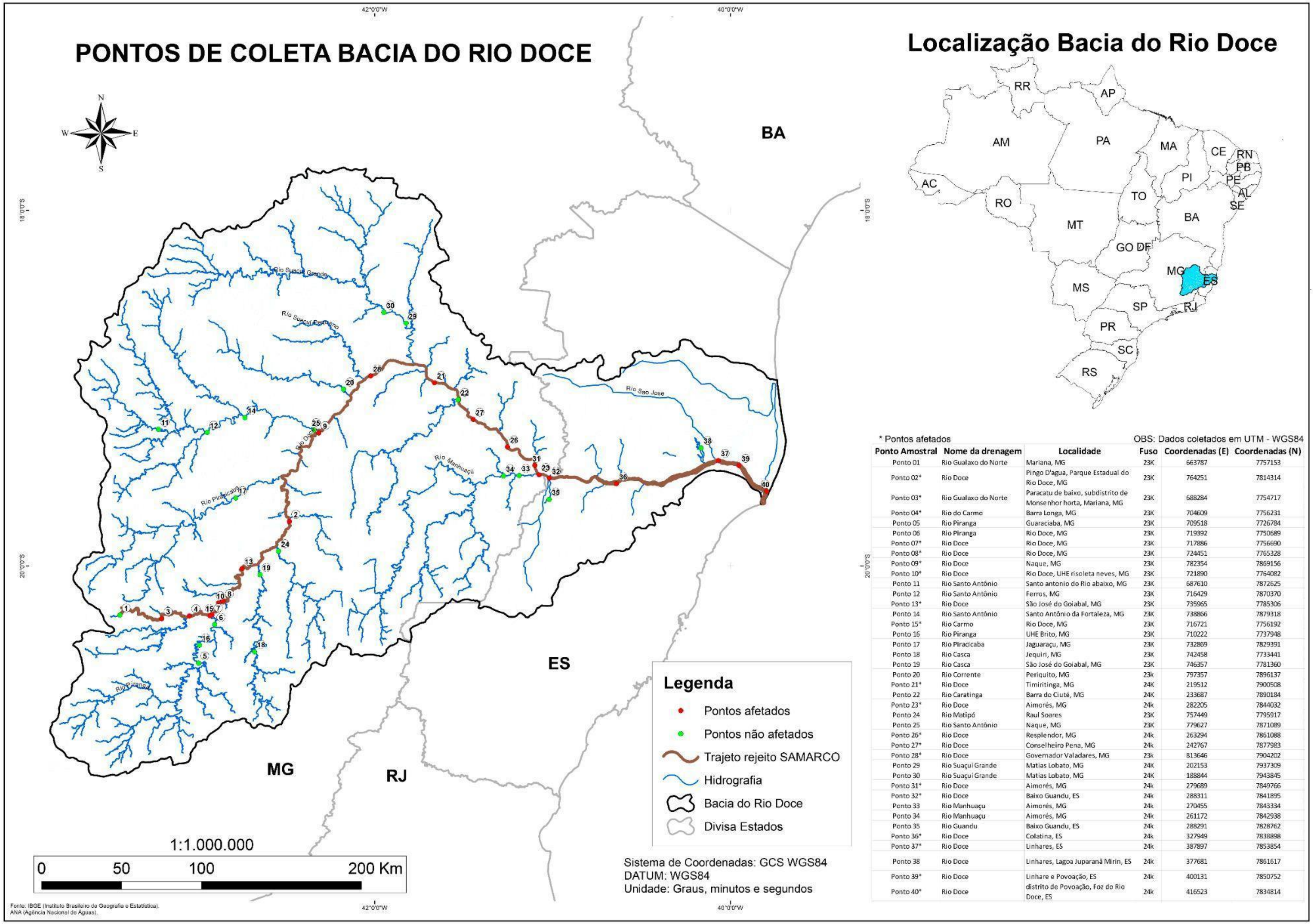
In addition, in the bioreduction of Fe and As, we detected an interaction of the ore tailings' effect with fish trophic level (Table 5): for Fe the ore tailings reduced the slope of bioreduction (Figure 25), while for As, the ore tailings increased the slope of bioreduction (Figure 11). This result indicates that the ore tailings changed the bioavailability of arsenic, additionally to the historical processes of leaching of arsenic and iron from geological sites rich in these elements such as the headwaters of the Doce River basin (Vergilio et al 2020).

The interaction effects of the ore tailings with trophic level for Ag, Cu and Pb, (Table 5), show that the ore tailings altered the biomagnification processes, with apparent biomagnification of Ag in reference sites and bioreduction in affected sites (Figure 4), while for Cu and Pb, we observed bioreduction in unaffected sites and possible biomagnification unaffected sites (Figures 20 and 40). Our overall results on the effects of fish trophic level corroborate Gray's (2002) conclusion that biomagnification of metals rarely occurs in nature, except for mercury, countering Croteau et al. 's (2005) evidence of Cd bioaccumulation in fish.

CONCLUSION

Arsenic and Zn showed higher concentrations in fishes from sites that were affected by the ore tailings released with SAMARCO/BHP Billiton/Vale's mining dam rupture. The richness of these elements in the studied region and the physicochemical interaction with the ore tailings seem to be responsible for the differences in the concentrations of As and Zn in fish muscle tissue from affected sites. Some fish species are more sensitive to chemical element variations, such as *Hoplias intermedius* and *Loricariichthys castaneus*, and some elements are more bioavailable in the aquatic environment, such as As and Hg. We found significant bioaccumulation of Hg in five of the nine analyzed species, which is one of the most widespread and toxic trace elements in the biosphere, can cause neuromotor disturbances and neuropathies, normally related to gold extraction. Fishes from the Gualaxo do Norte River were the only ones with an average concentration of mercury higher than that established by ANVISA for human consumption. Mercury presents biomagnification in fish muscle tissue, both in affected and unaffected sites. Ore tailings effects modified the bioaccumulation processes, reinforcing the evidence for physicochemical interaction of the ore tailings with the bioavailability of metals and their accumulation in the aquatic biota. The present study is not only an important step towards understanding the chronic impacts of the Fundão dam failure on the Doce River aquatic biota, but also an important contribution to the understanding of metals and Arsenic accumulation in fishes.

FIGURES



* Pontos afetados

OBS: Dados coletados em UTM - WGS84

Ponto Amostral	Nome da drenagem	Localidade	Fuso	Coordenadas (E)	Coordenadas (N)
Ponto 01	Rio Gualaxo do Norte	Mariana, MG	23K	663787	7757153
Ponto 02*	Rio Doce	Pingo D'agua, Parque Estadual do Rio Doce, MG	23K	764251	7814314
Ponto 03*	Rio Gualaxo do Norte	Paracatu de baixo, subdistrito de Monsenhor horta, Mariana, MG	23K	688284	7754717
Ponto 04*	Rio do Carmo	Barra Longa, MG	23K	704609	7756231
Ponto 05	Rio Piranga	Guaraciaba, MG	23K	709518	7726784
Ponto 06	Rio Piranga	Rio Doce, MG	23K	719392	7750689
Ponto 07*	Rio Doce	Rio Doce, MG	23K	717886	7756690
Ponto 08*	Rio Doce	Rio Doce, MG	23K	724451	7765328
Ponto 09*	Rio Doce	Naque, MG	23K	782354	7869156
Ponto 10*	Rio Doce	Rio Doce, UHE risoleta neves, MG	23K	721890	7764082
Ponto 11	Rio Santo Antônio	Santo antonio do Rio abaixo, MG	23K	687610	7872625
Ponto 12	Rio Santo Antônio	Ferros, MG	23K	716429	7870370
Ponto 13*	Rio Doce	São José do Goiabal, MG	23K	735965	7785306
Ponto 14	Rio Santo Antônio	Santo Antônio da Fortaleza, MG	23K	738866	7879318
Ponto 15*	Rio Carmo	Rio Doce, MG	23K	716721	7756192
Ponto 16	Rio Piranga	UHE Brito, MG	23K	710222	7737948
Ponto 17	Rio Piracicaba	Jaguaraçu, MG	23K	732869	7829391
Ponto 18	Rio Casca	Jequiri, MG	23K	742458	7733441
Ponto 19	Rio Casca	São José do Goiabal, MG	23K	746357	7781360
Ponto 20	Rio Corrente	Periquito, MG	23k	797357	7896137
Ponto 21*	Rio Doce	Timiritinga, MG	24K	219512	7900508
Ponto 22	Rio Caratinga	Barra do Ciuté, MG	24K	233687	7890184
Ponto 23*	Rio Doce	Aimorés, MG	24k	282205	7844032
Ponto 24	Rio Matipó	Raul Soares	23K	757449	7795917
Ponto 25	Rio Santo Antônio	Naque, MG	23K	779627	7871089
Ponto 26*	Rio Doce	Resplendor, MG	24k	263294	7861088
Ponto 27*	Rio Doce	Conselheiro Pena, MG	24k	242767	7877983
Ponto 28*	Rio Doce	Governador Valadares, MG	23k	813646	7904202
Ponto 29	Rio Suaçuí Grande	Matias Lobato, MG	24K	202153	7937309
Ponto 30	Rio Suaçuí Grande	Matias Lobato, MG	24K	188844	7943845
Ponto 31*	Rio Doce	Aimorés, MG	24k	279689	7849766
Ponto 32*	Rio Doce	Baixo Guandu, ES	24k	288311	7841895
Ponto 33	Rio Manhuaçu	Aimorés, MG	24k	270455	7843334
Ponto 34	Rio Manhuaçu	Aimorés, MG	24k	261172	7842938
Ponto 35	Rio Guandu	Baixo Guandu, ES	24k	288291	7828762
Ponto 36*	Rio Doce	Colatina, ES	24k	327949	7838898
Ponto 37*	Rio Doce	Linhares, ES	24k	387897	7853854
Ponto 38	Rio Doce	Linhares, Lagoa Juparanã Mirin, ES	24k	377681	7861617
Ponto 39*	Rio Doce	Linhare e Povoação, ES	24k	400131	7850752
Ponto 40*	Rio Doce	distrito de Povoação, Foz do Rio Doce, ES	24k	416523	7834814

Ag — Silver

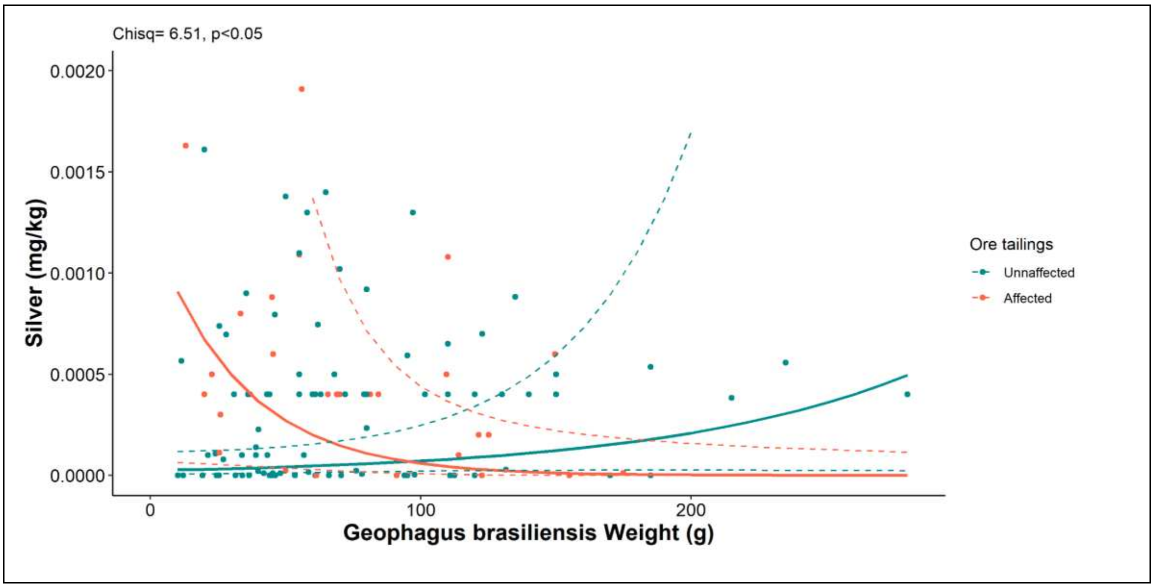


Figure 2. Concentration of silver (Ag) in *Geophagus brasiliensis* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). While silver concentration increased with fish weight (bioaccumulation) in unaffected sites, affected sites this process was reversed (biodilution) (χ^2=6.51; p=0.0107).

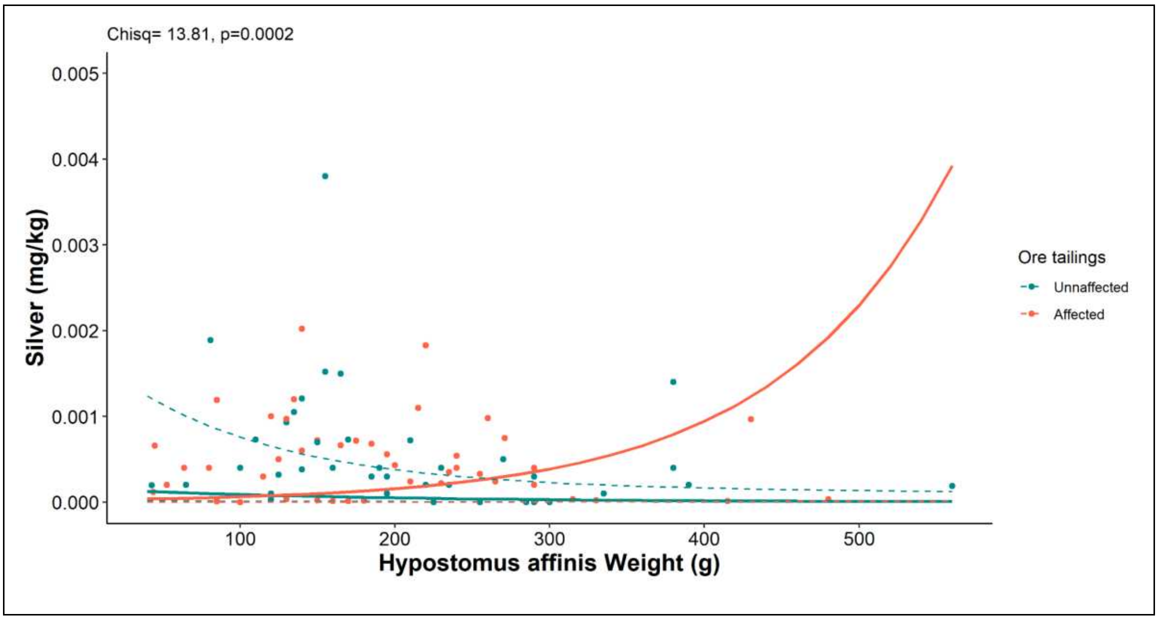


Figure 3: Concentration of silver (Ag) in *Hypostomus affinis* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). While silver concentration decreased with fish weight (biodilution) in unaffected sites, affected sites this process was reversed (bioaccumulation) (χ2=13.81; p=0.0002).

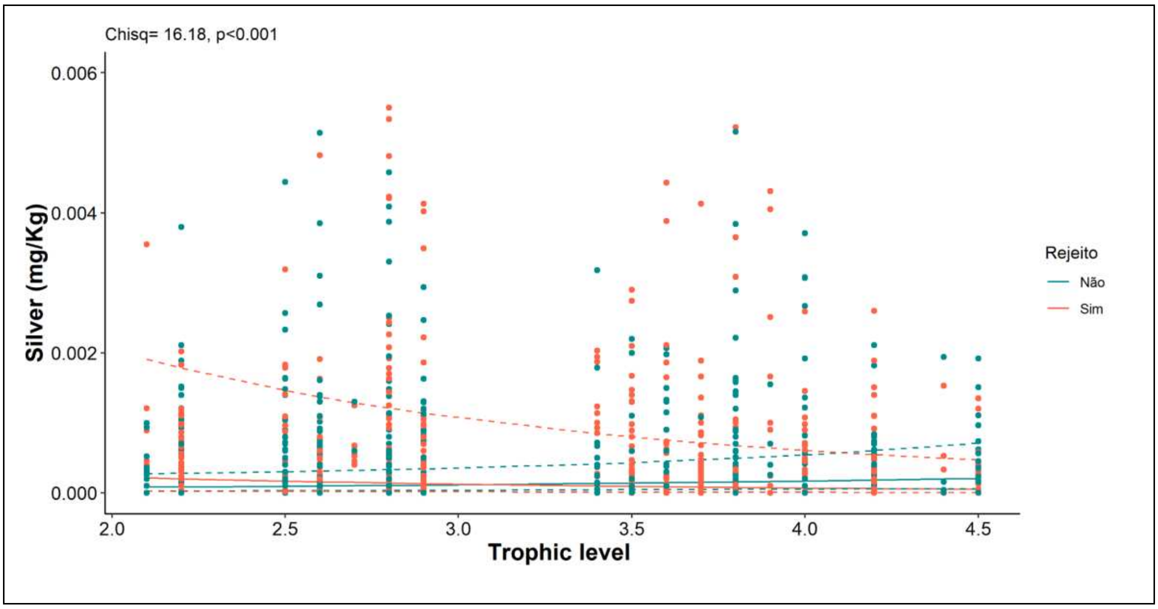


Figure 4: Silver (Ag) concentration in muscle tissue in relation to the trophic level of fish. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). While silver concentration decreased with fish weight (biodilution) in affected sites, unaffected sites this process was reversed (bioaccumulation) ($\chi 2$=16.18; p= 5.73 e^{-5}).

Al — Aluminum

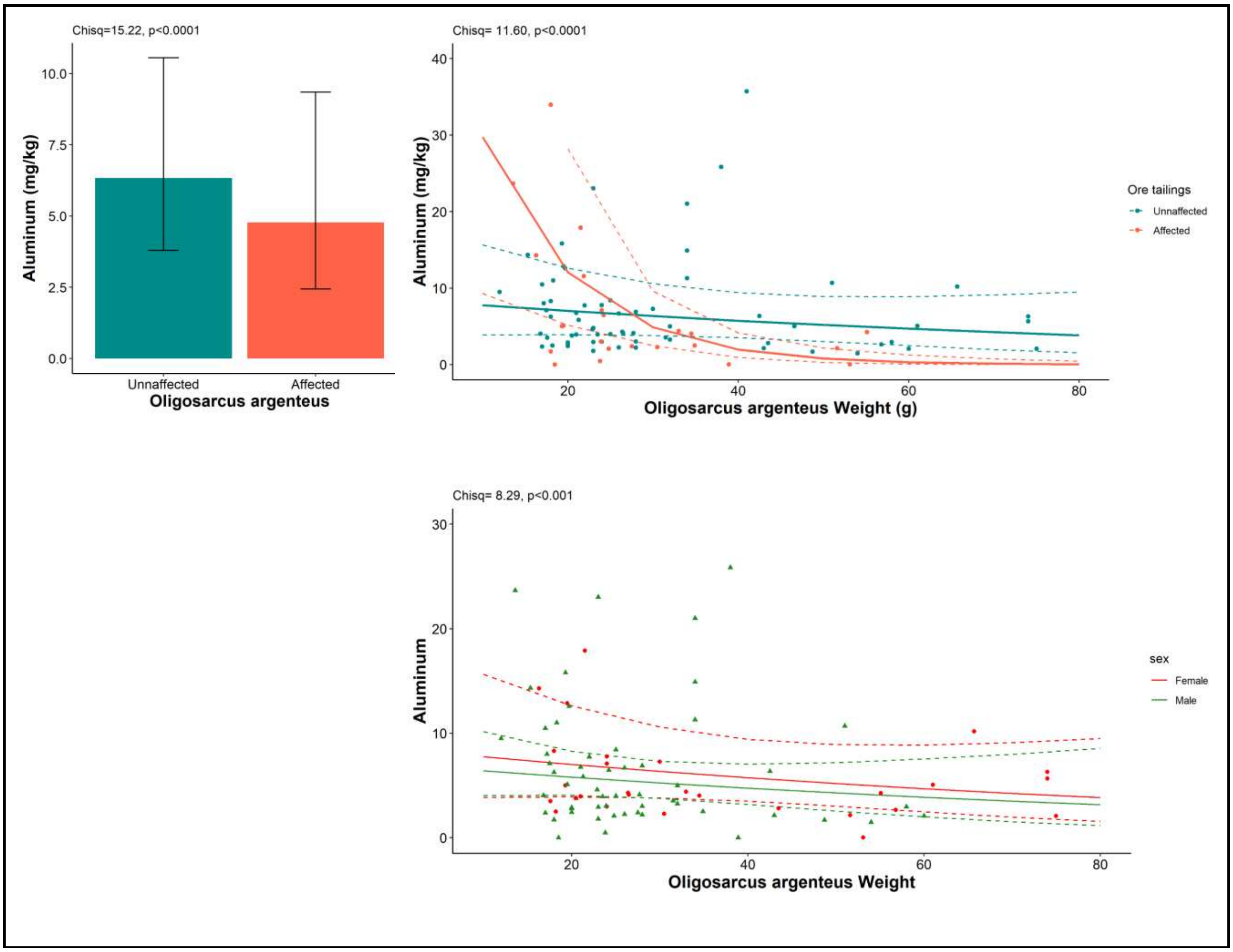


Figure 5: (A) Concentration of aluminum (Al) in *Oligosarcus argenteus* muscle tissue. Green bar: fishes in unaffected sites; orange bar: fishes in affected sites; aluminum concentration was lower among fish from affected sites compared to fish from unaffected sites (χ^2=15.22, p=9.56 e^{-5}). **(B)** There was biodilution of aluminum in *O. argenteus* (χ^2=6.93, p=0.0084) and also an interaction between the effects of weight and tailings (χ^2=11.60, p=0.0006) indicating that the presence of the ore tailings affected *O. argenteus'* biodilution processes. **(C)** *O. argenteus* female fishes presented higher concentrations of aluminum than male fishes (χ^2=6.21, p=0.0126), with an interaction, despite subtle, between the effects of sex and weight (χ^2=8.29, p=0.0039), showing that the bioaccumulation process differed between female and male *O. argenteus*. Green dots: Male fishes; red dots: Female fishes; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

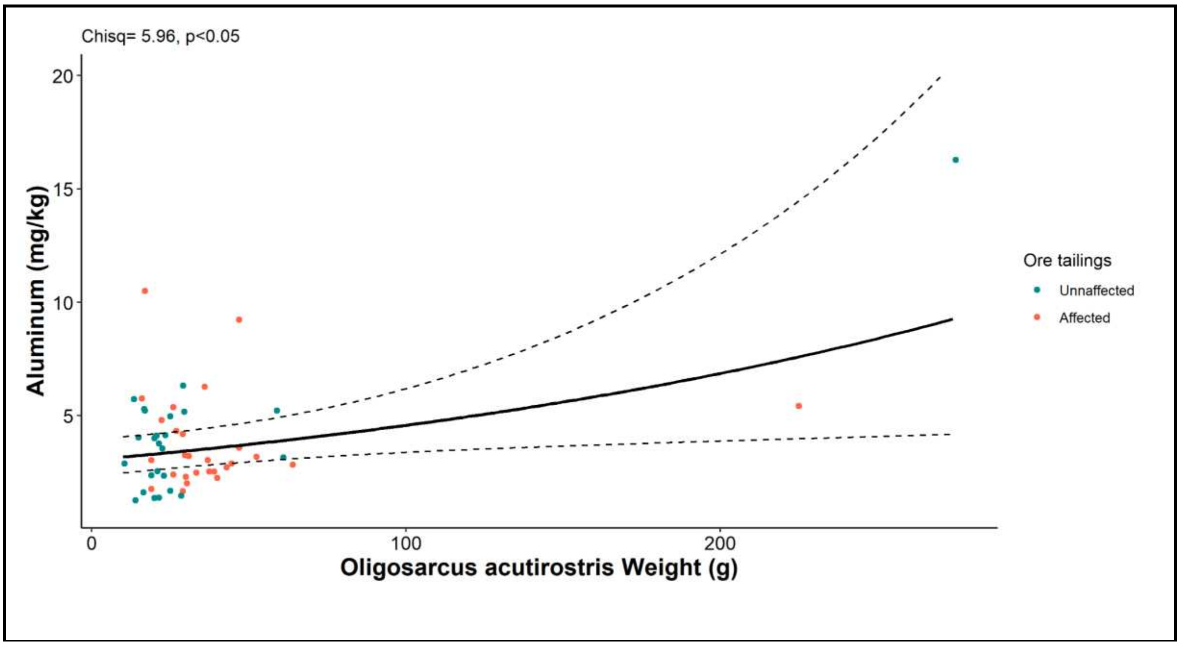


Figure 6: Concentration of aluminum (Al) in *Oligosarcus acutirostris* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). There was bioaccumulation of aluminum in *Oligosarcus acutirostris* in both affected and unaffected sites (=5.96, p=0.0195).

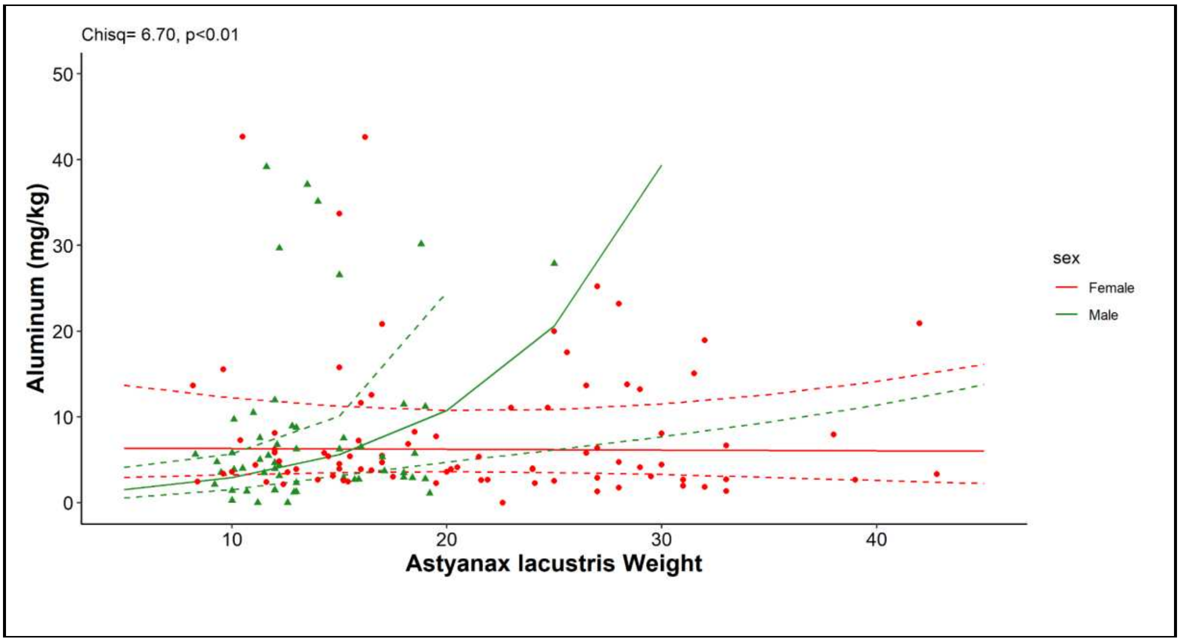


Figure 7: Concentration of aluminum (Al) in *Astyanax lacustris* muscle tissue. Green dots: Male fishes; red dots: Female fishes; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). Bioaccumulation processes differed between females and males of *Astyanax lacustris* (χ^2=6.70, p=0.0095), with bioaccumulation in males, but not in females, in both affected and unaffected sites.

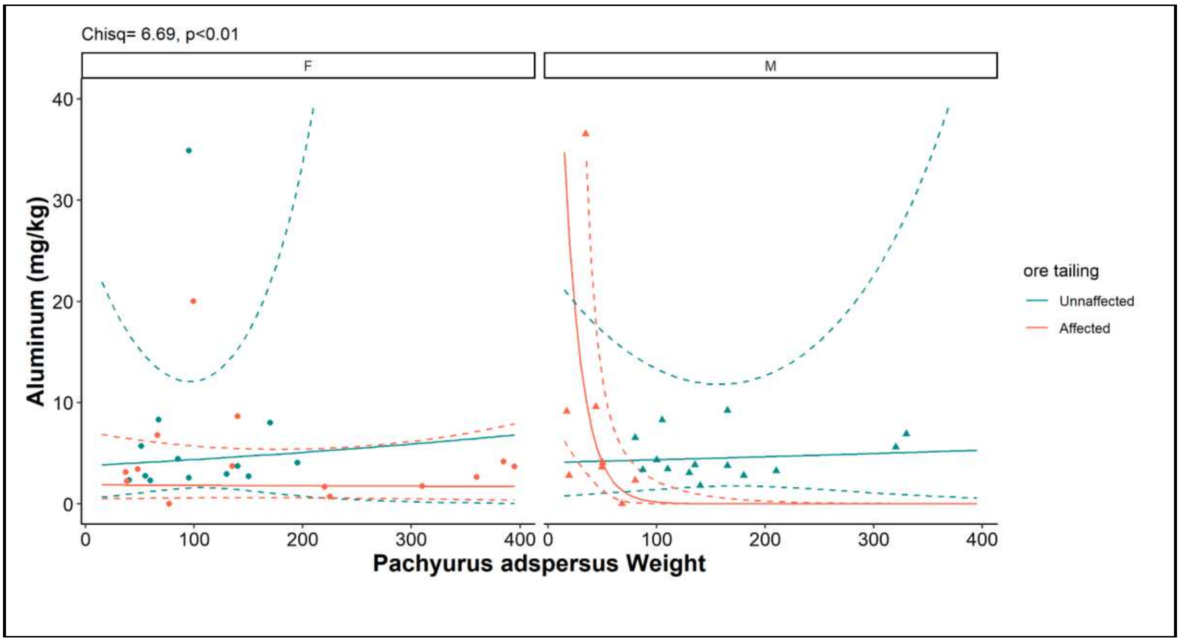


Figure 8: Concentration of aluminum (Al) in *Pachyurus adspersus* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). *P. adspersus* showed interaction between the effects of ore tailings, weight and sex, indicating differences in the bioaccumulation processes between male and female fish from affected and unaffected sites (χ^2=6.69, p=0.0082).

As — Arsenic

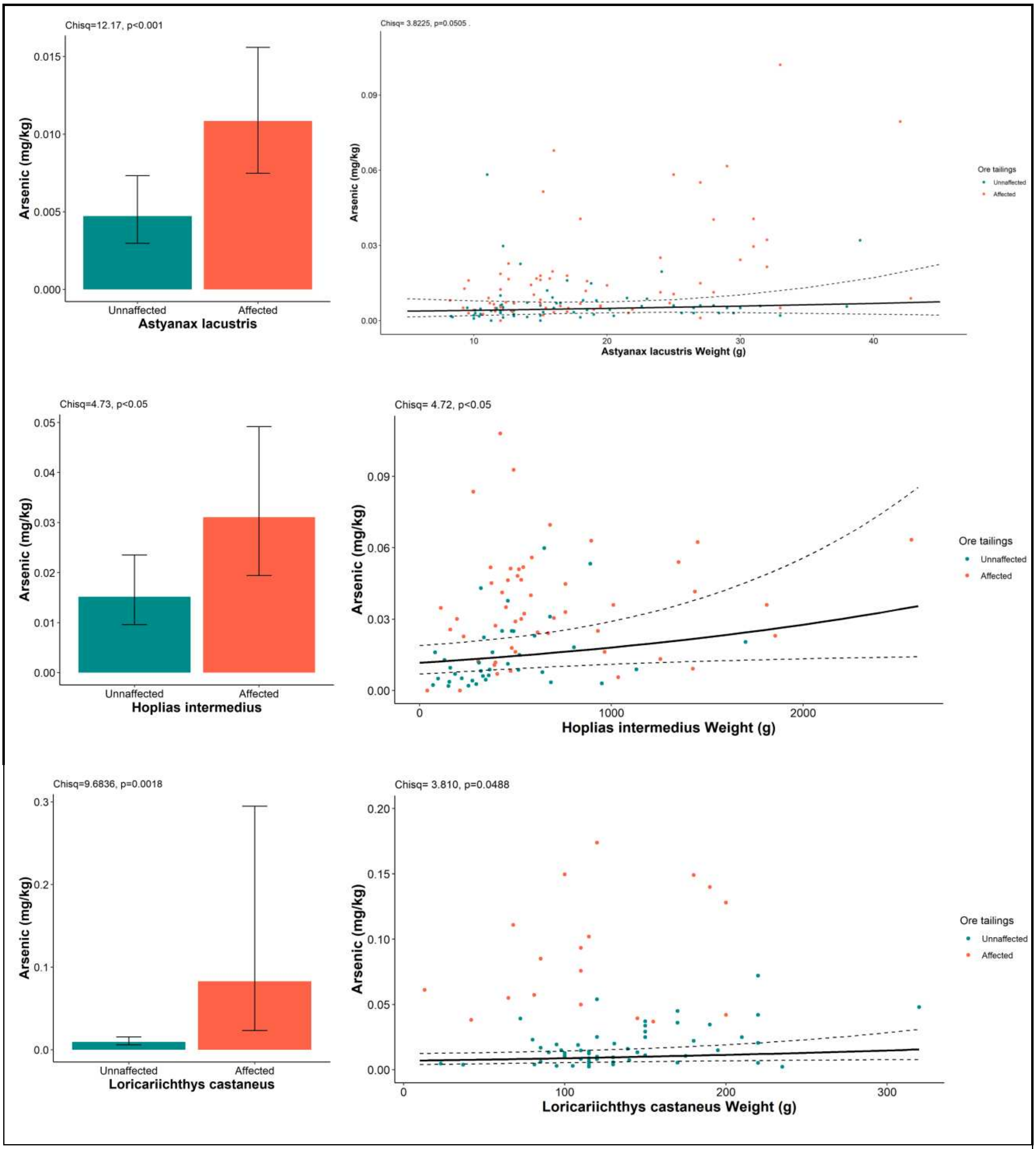


Figure 9: The arsenic concentration in muscle tissue was higher among fish from affected sites compared to fish from unaffected sites and there was bioaccumulation of arsenic in three species: **(A)** *Astyanax lacustris* (χ^2=12.17, p=0.0004), **(B)** *A. lacustris* (bioaccumulation/ χ^2=3.82, p=0.0505), **(C)** *Hoplias intermedius* (χ^2=4.73, p=0.0295), **(D)** *H. intermedius* (bioaccumulation/ χ^2=4.72, p=0.0297), **(E)** *Loricariichthys castaneus* (χ^2=9.68, p=0.005), **(F)** *L. castaneus* (bioaccumulation/ χ^2=3.81, p=0.0488). Green bar: arsenic concentration in fishes from unaffected sites; orange bar: arsenic concentration in fishes from affected sites; Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

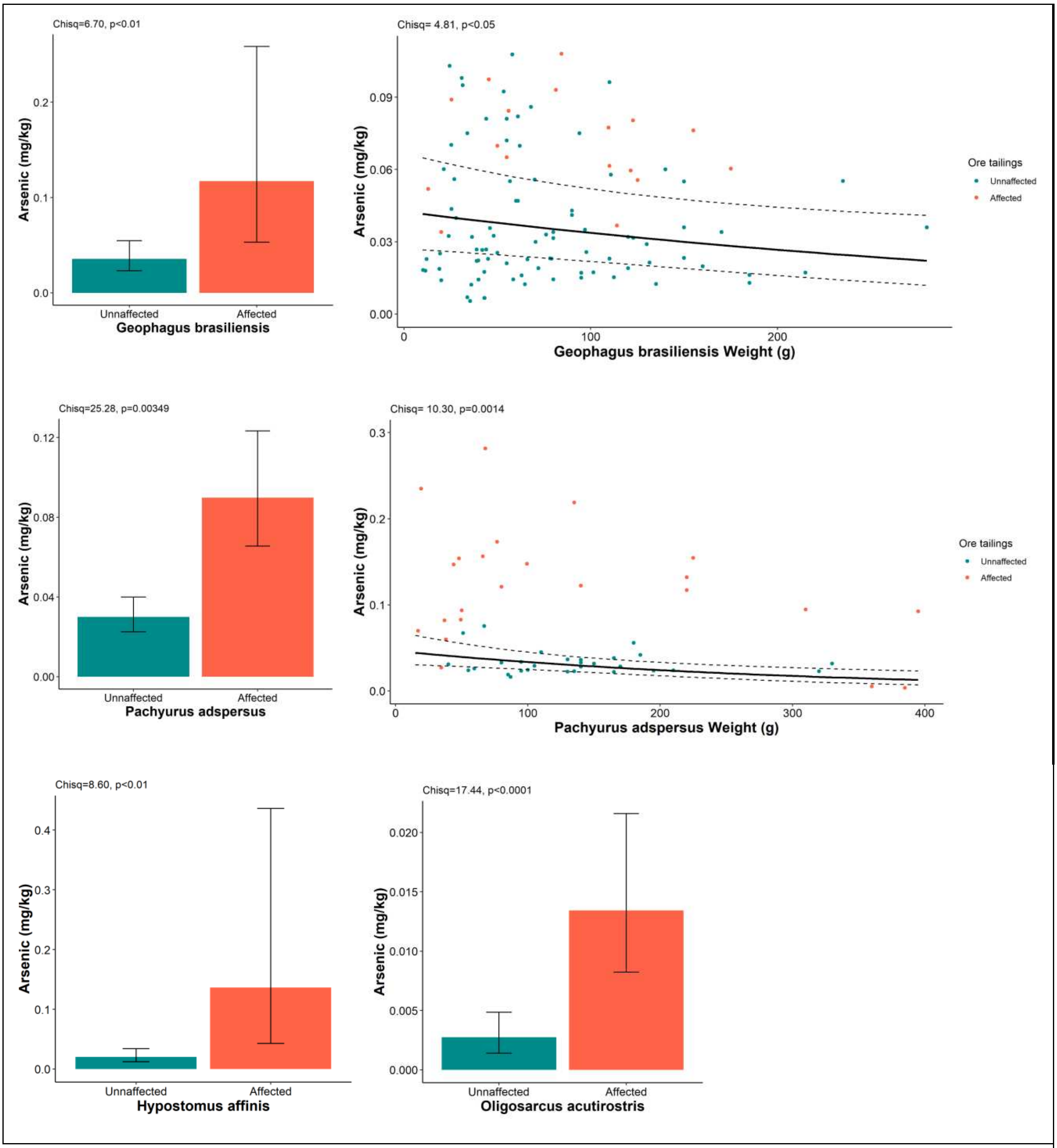


Figure 10: The arsenic concentration in muscle tissue was higher among fish from affected sites compared to fish from unaffected sites and there was biodilution of arsenic in two species: (**A**) *Geophagus brasiliensis* (χ^2=6.70, p=0.0096), **(B)** *G. brasiliensis* (biodilution / χ^2=4.81, p=0.0282), **(C)** *Pachyurus adspersus* (χ^2=25.28, p=0.0035), **(D)** *P. adspersus* (biodilution / χ^2=10.30, p=0.0014). There was no relation between arsenic concentration and fish weight in **(E)** *Hypostomus affinis* (χ^2=8.60, p=0.0061), **(F)** *Oligosarcus acutirostris* (χ^2=17.44, p=2.96 e^{-5}). Green bar: arsenic concentration in fishes from unaffected sites; orange bar: arsenic concentration in fishes from affected sites; Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

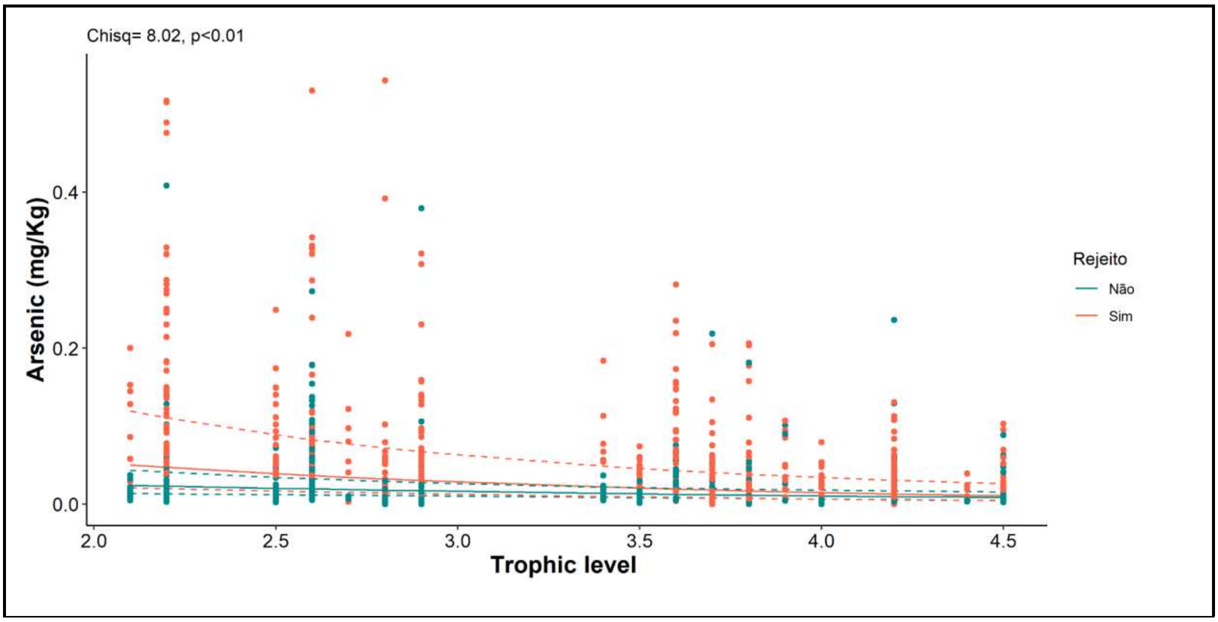
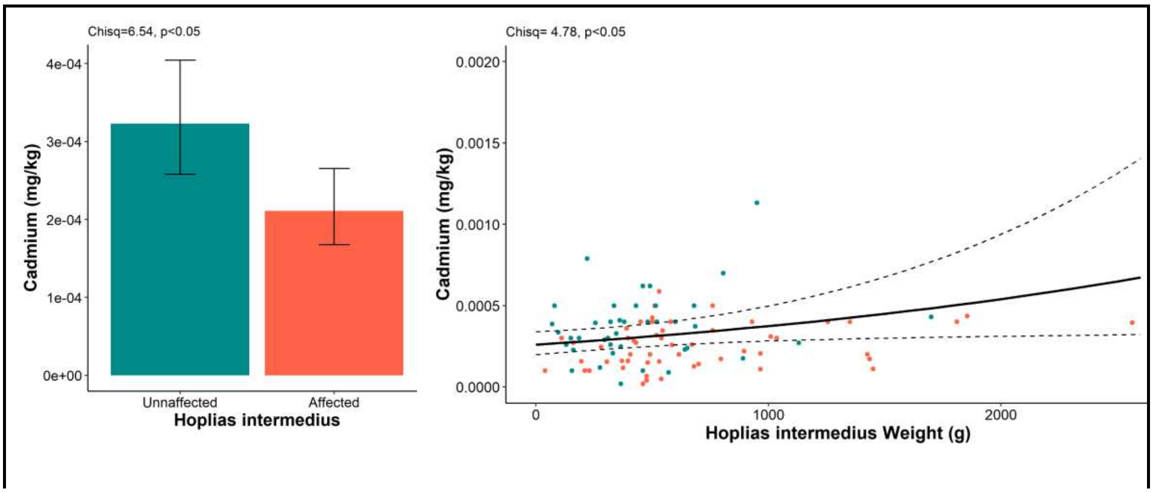


Figure 11: Arsenic (As) concentration in muscle tissue in relation to the trophic level of fish. The concentration decreased along trophic levels (bioreduction) (χ^2=11.74, p=0.0006) and there was an interaction between the effect tailings and of trophic level: the effects of tailings were higher at lower trophic levels (χ^2=8.02, p=0.0046). Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

Cd — Cadmium



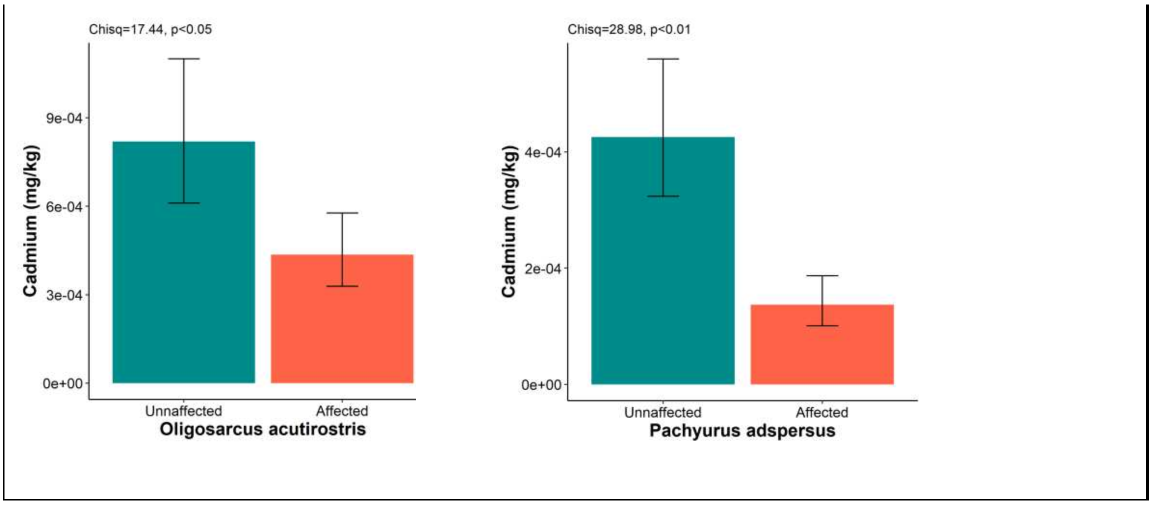


Figure 12: Cadmium concentrations were lower among fish from affected sites compared to fish from unaffected sites in three studied species: **(A)** *Hoplias intermedius* (χ^2=6.54, p=0.0472), **(C)** *Oligosarcus acutirostris* (χ^2=17.44, p=0.0103), and **(D)** *Pachyurus adspersus* (χ^2=28.98, p=7.30 e^{-8}). **(B)** There was bioaccumulation of cadmium only in *Hoplias intermedius* (bioaccumulation/ χ^2=4.78, p=0.0287). Green bar: cadmium concentration in fishes from unaffected sites; orange bar: cadmium concentration in fishes from affected sites; Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

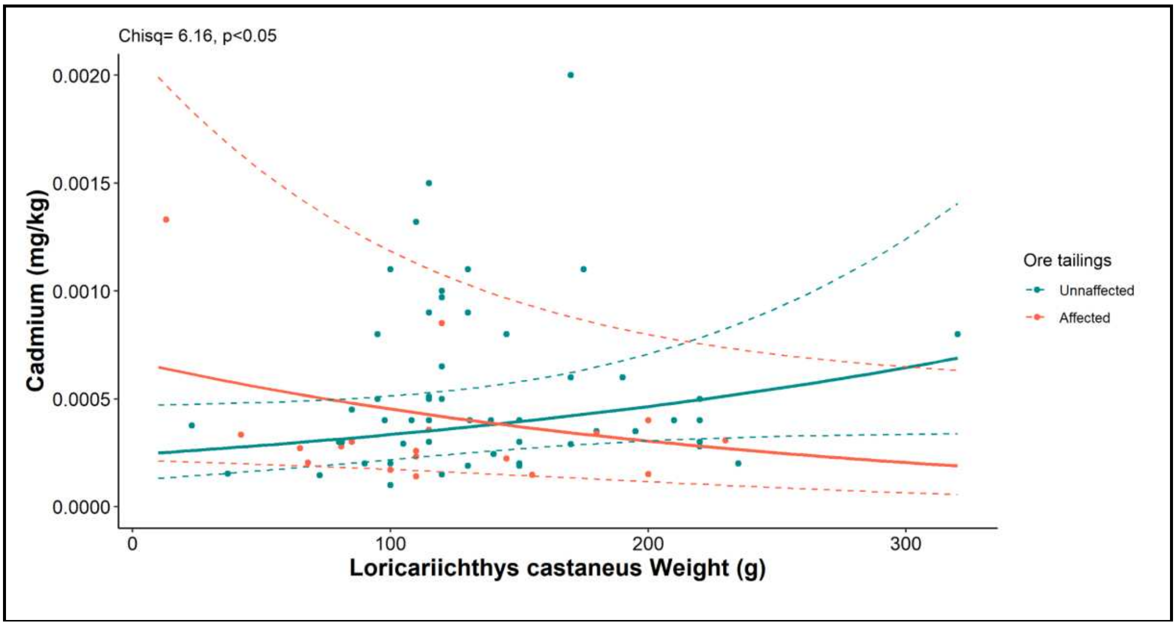


Figure 13: Concentration of cadmium (Cd) in *Loricariichthys castaneus* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). While cadmium concentration increased with fish weight (bioaccumulation) in unaffected sites, affected sites this process was reversed (biodilution) (χ^2=6.16; p=0.013).

Cr — Chromium

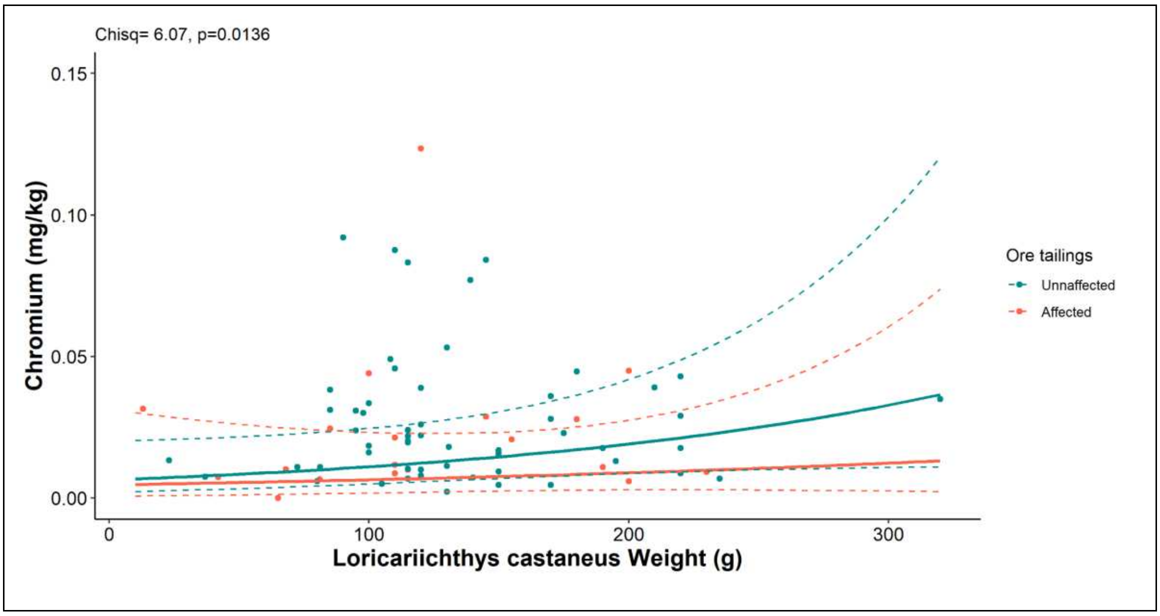


Figure 14: Concentration of chromium (Cr) in *Loricariichthys castaneus* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). Chromium concentration increased with fish weight (bioaccumulation) in *Loricariichthys castaneus in* both unaffected and affected sites (χ^2=3.90, p=0.0482).

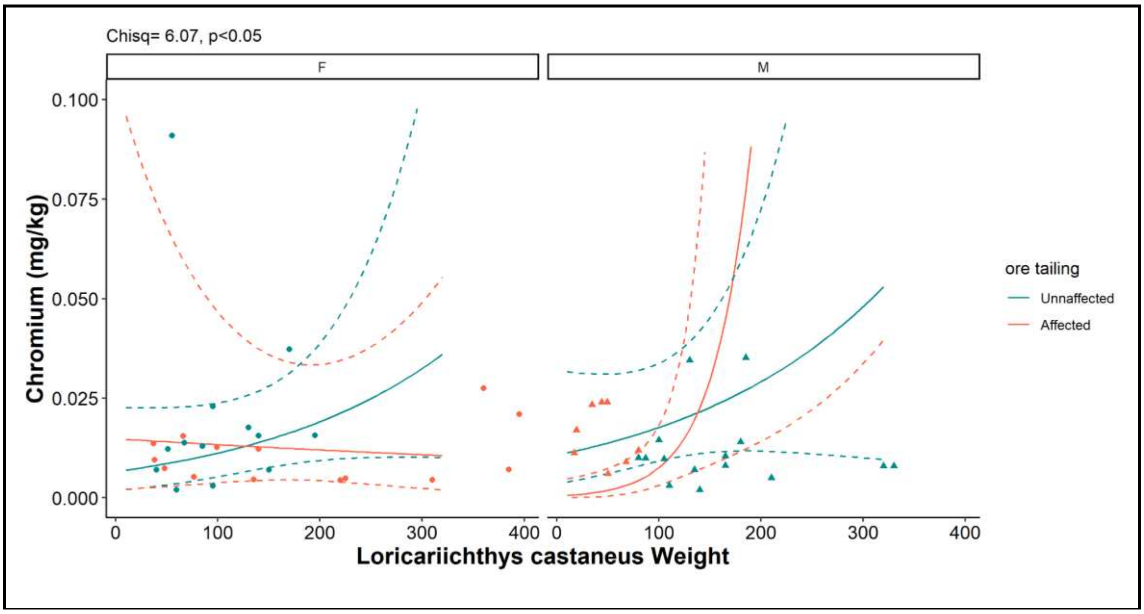


Figure 15: Concentration of chromium (Cr) in *Loricariichthys castaneus* muscle tissue. *L. castaneus* showed interaction between the effects of ore tailings, weight and sex, indicating differences in the bioaccumulation processes between male and female fish from affected and unaffected sites (χ^2=6.07, p=0.0136). Females in affected sites presented evidence of biodilution. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

Cu — Copper

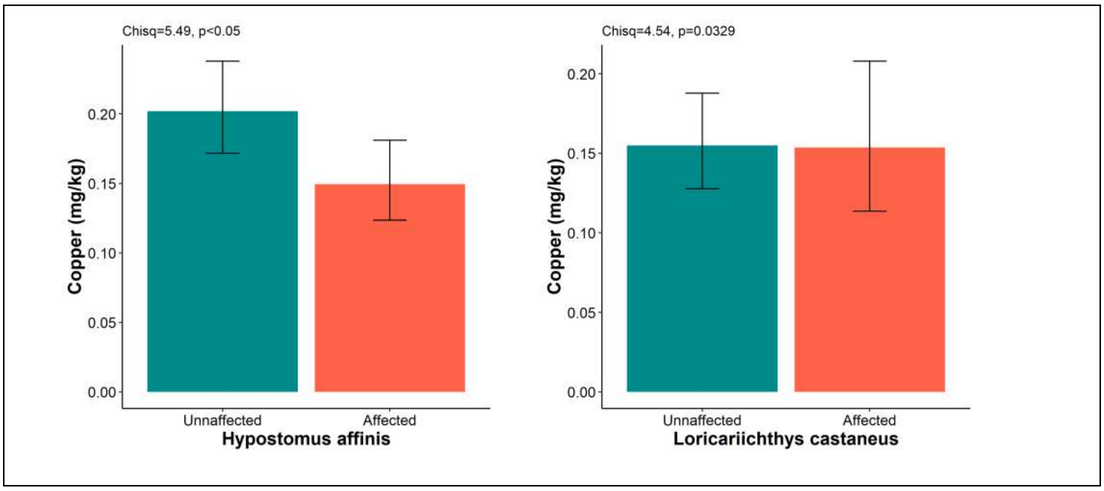


Figure 16: Copper (Cu) concentrations were lower among fish from affected sites compared to fish from unaffected sites in two studied species: (A) *Hypostomus affinis* (χ^2=5.49, p=0.0340) and (B) *Loricariichthys castaneus* (χ^2=4.54, p=0.0329). Green bar: copper concentration in fishes from unaffected sites; orange bar: copper concentration in fishes from affected sites; Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

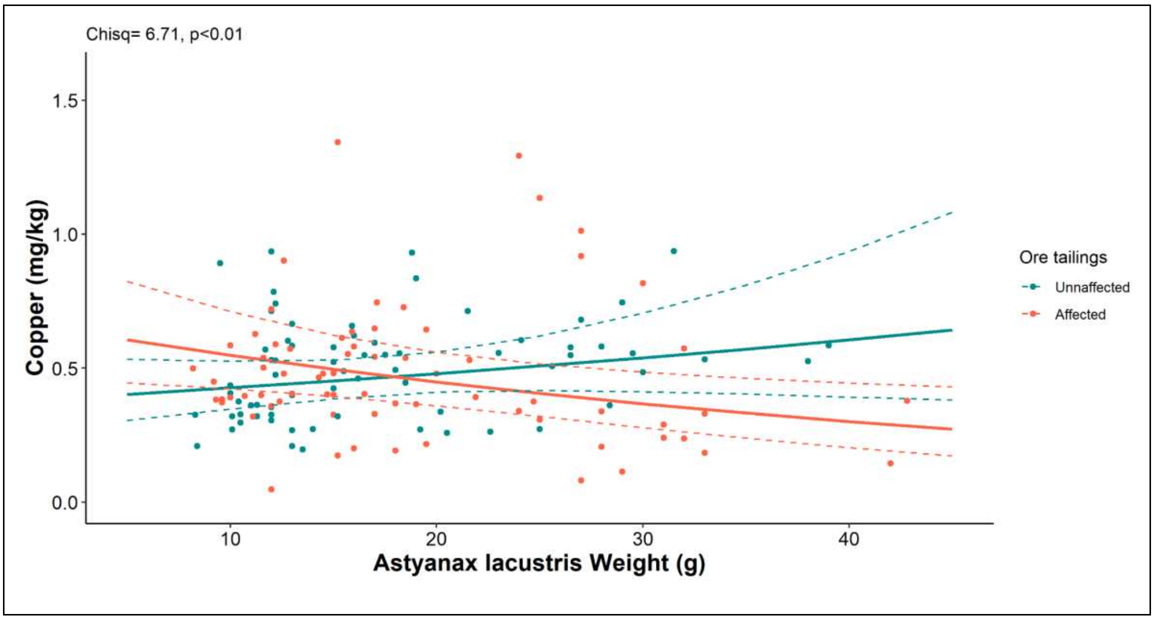


Figure 17: Concentration of copper (Cu) in *Astyanax lacustris* muscle tissue. While copper concentration increased with fish weight (bioaccumulation) in unaffected sites, affected sites this process was reversed (biodilution) (χ^2=6.71, p=0.0121). Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the

minimum adequate model (GLMM).

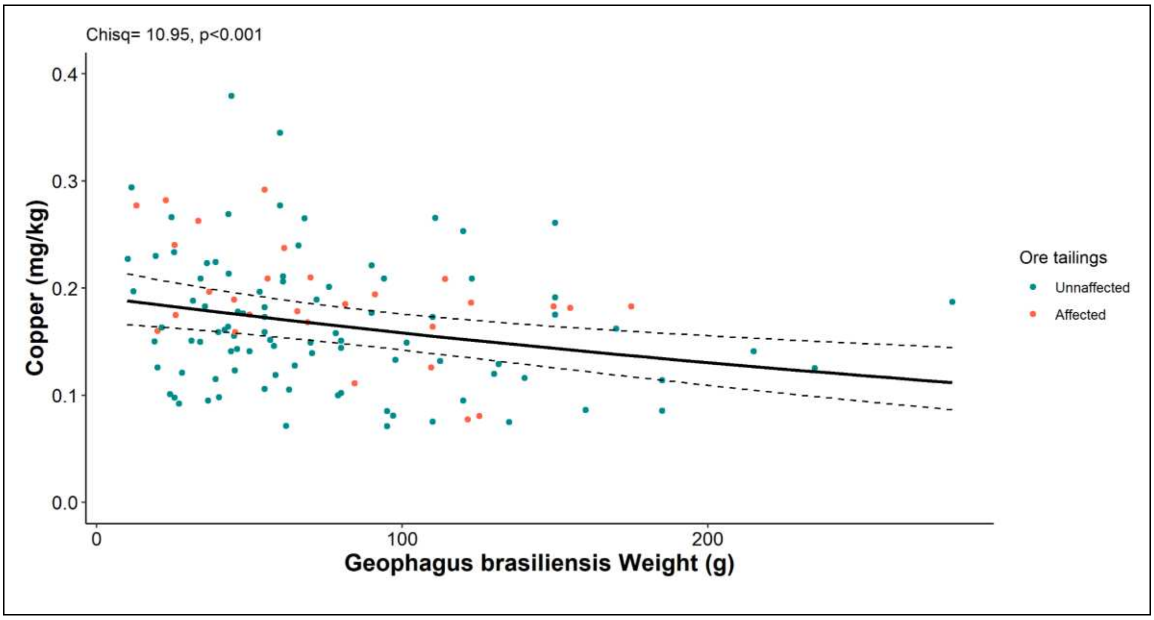


Figure 18: Concentration of copper (Cu) in *Geophagus brasiliensis* muscle tissue. There was biodilution of copper in *G. brasiliensis* in both affected and unaffected sites (χ^2=10.95, p=0.0009). Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

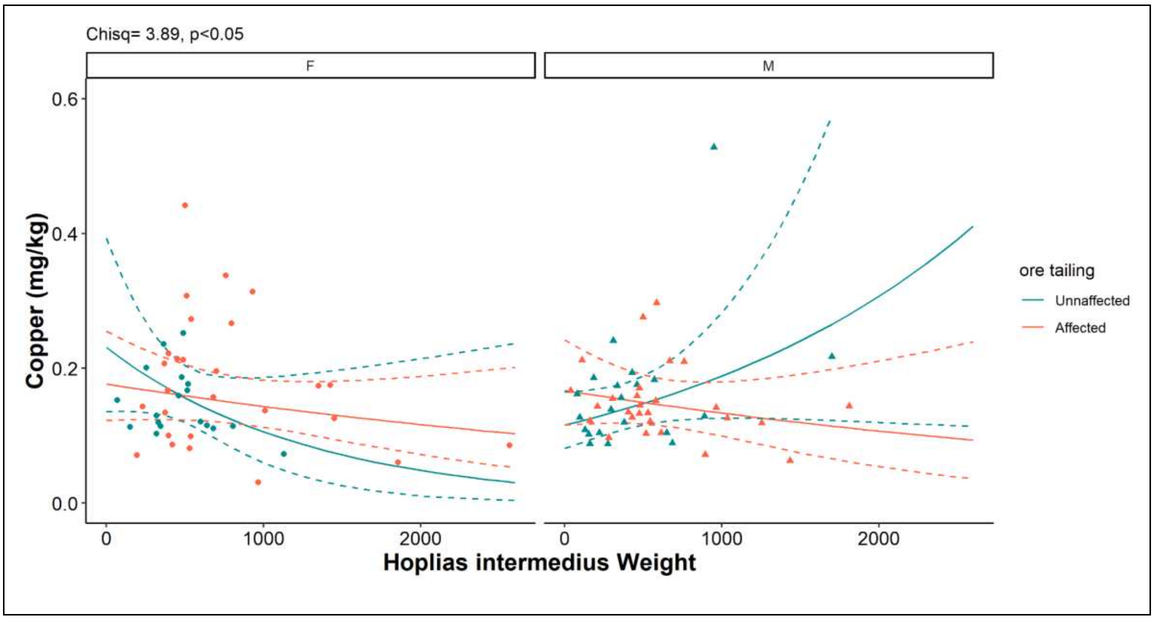


Figure 19: Concentration of copper (Cu) in *Hoplias intermedius* muscle tissue. *H. intermedius* showed interaction between the effects of ore tailings, weight and sex, indicating differences in the bioaccumulation processes between male and female fish from affected and unaffected sites

(χ^2=3.89, p=0.0483). There was a biodilution in female fishes from affected and unaffected sites and in male fishes from affected sites, but males in unaffected sites presented evidence of bioaccumulation. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

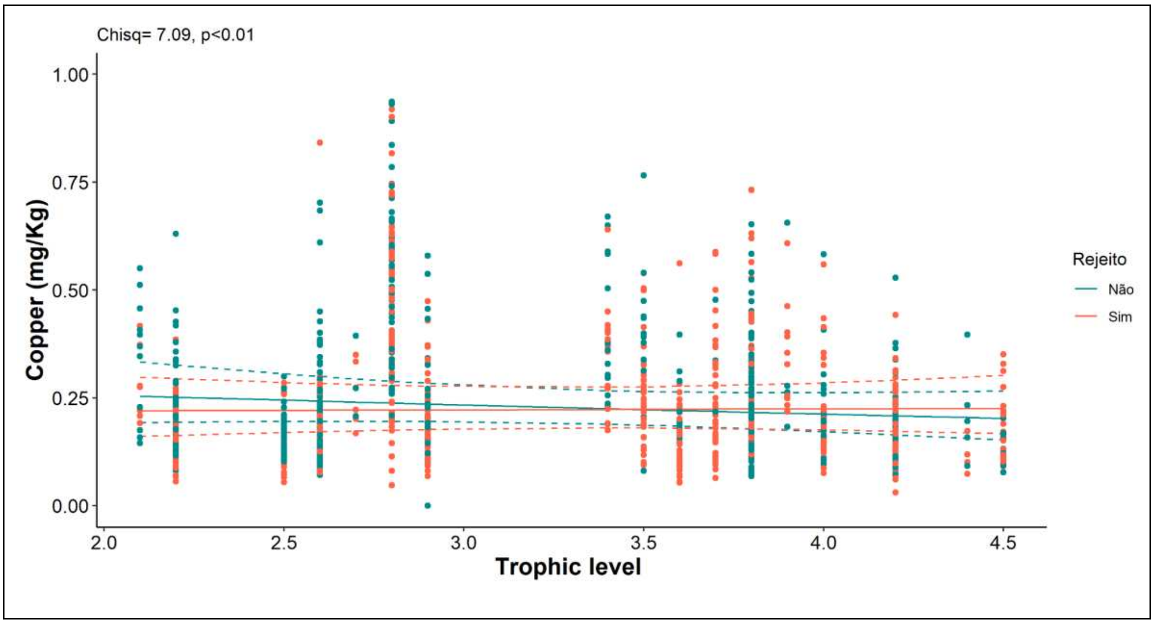


Figure 20: Copper (Cu) concentration in muscle tissue in relation to the trophic level of fish. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). Copper concentration did not increase along trophic levels, thus discarding any evidence for biomagnification of copper, but an interaction was detected between the effects of tailings and trophic level, showing increase in copper concentrations with trophic level (biomagnification) in the presence of tailings, contreasting with a decrease in copper concentrations with trophic level (bioreduction) in unaffected sites (χ^2=7.09, p= 0.0077).

Fe — Iron

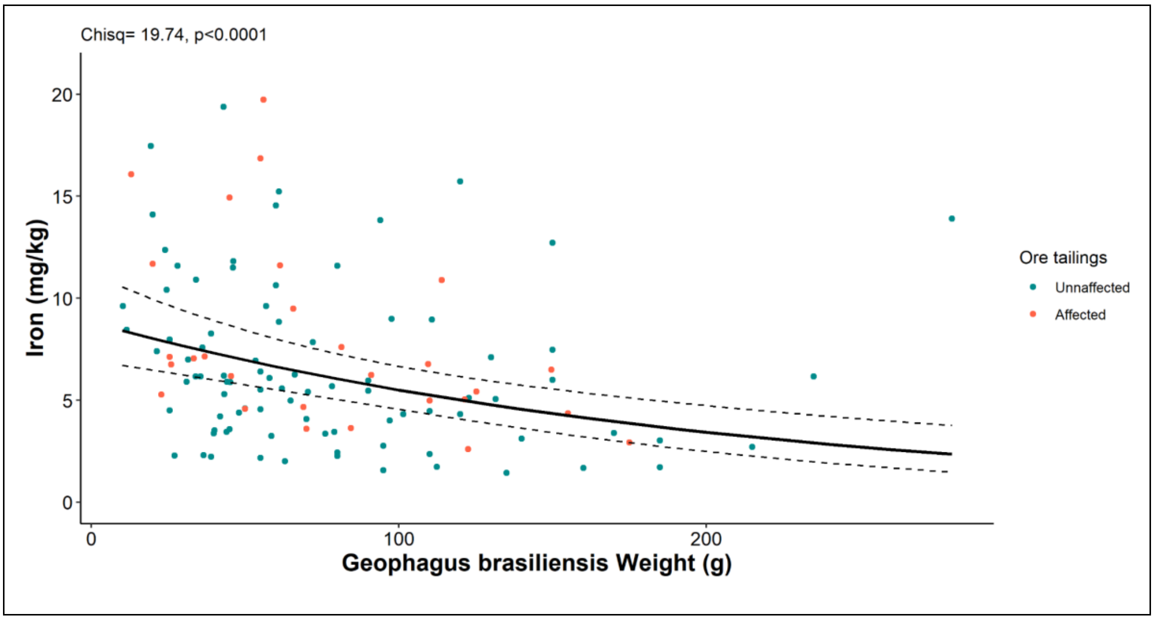


Figure 21: Concentration of iron (Fe) in *Geophagus brasiliensis* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). There was biodilution of iron in *G. brasiliensis* in both affected and unaffected sites (χ^2=19.74, p=2.11e^{-5}).

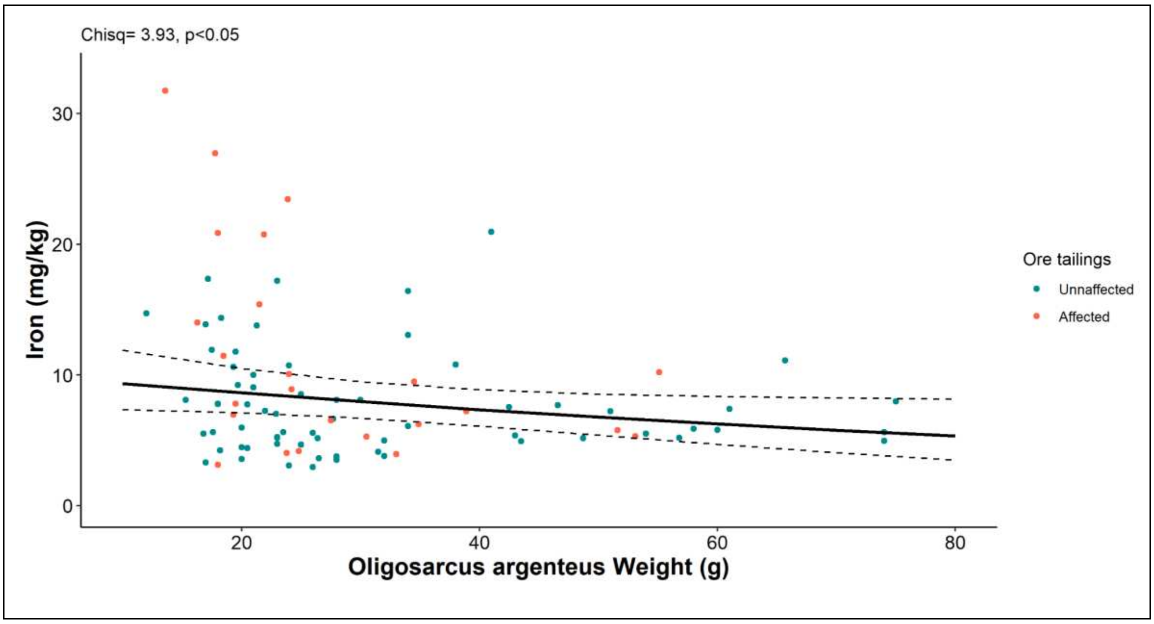


Figure 22: Concentration of iron (Fe) in *Oligosarcus argenteus* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). There was biodilution of iron in *O. argenteus* in both affected and unaffected sites (χ^2=3.93, p=0.0473).

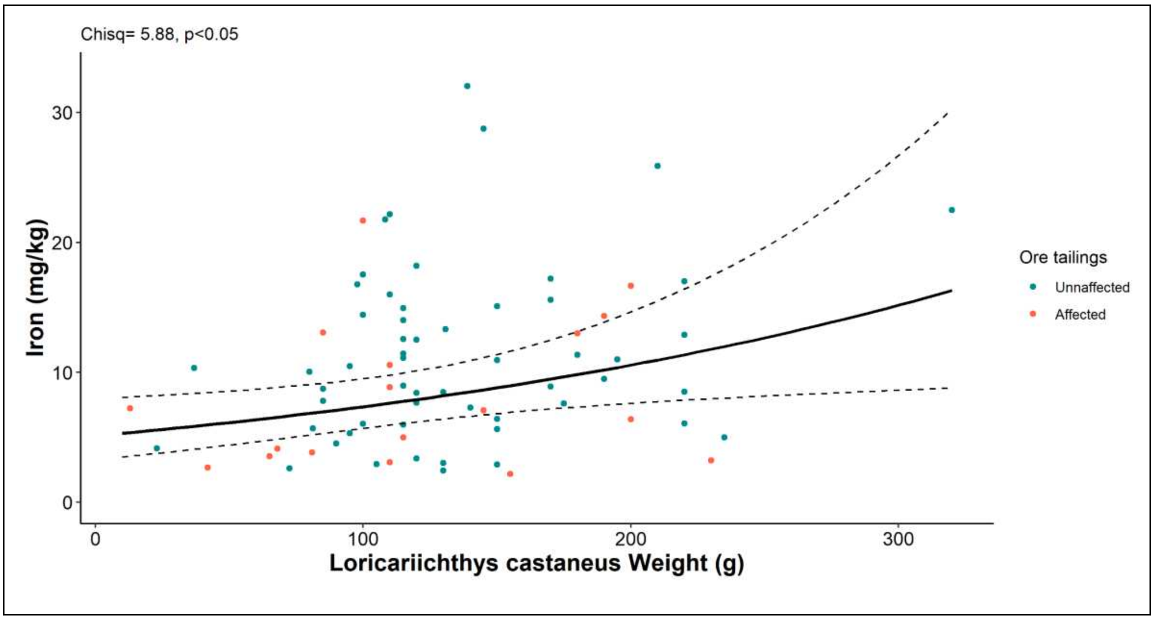


Figure 23: Concentration of iron (Fe) in *Loricariichthys castaneus* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). There was biodilution of iron in *L. castaneus* in both affected and unaffected sites (χ^2=5.88, p=0.0162).

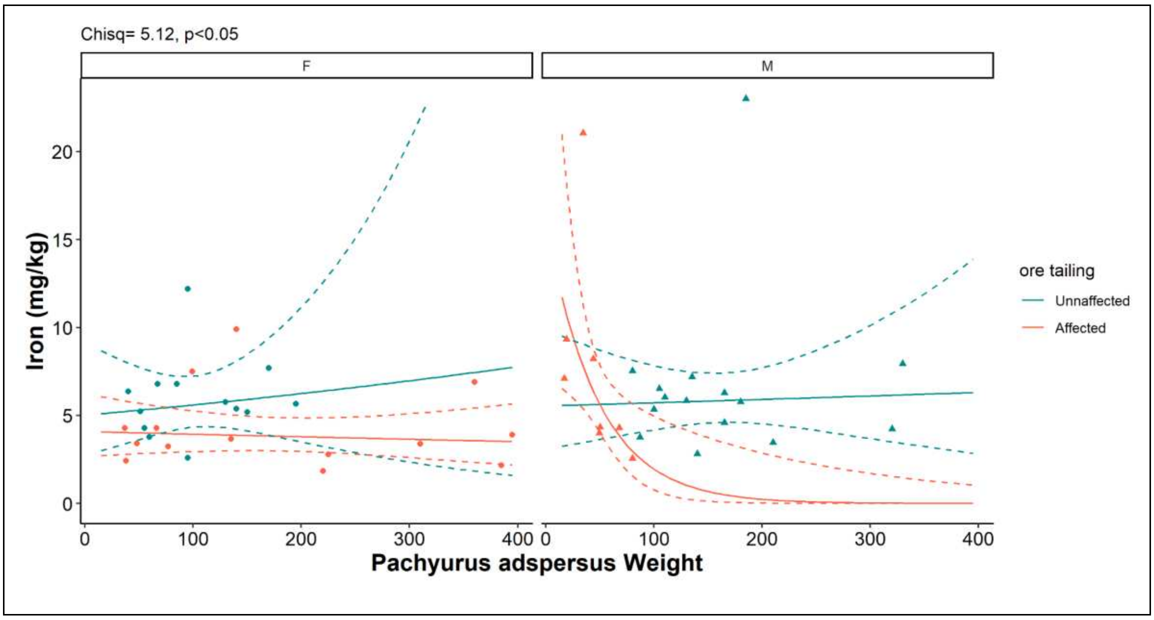


Figure 24: Concentration of iron (Fe) in *Pachyurus adspersus* muscle tissue. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). *P. adspersus* showed interaction between the effects of ore tailings, weight and sex, indicating differences in the bioaccumulation processes between male and female fish from affected and unaffected sites (χ^2=5.12, p=0.0136).

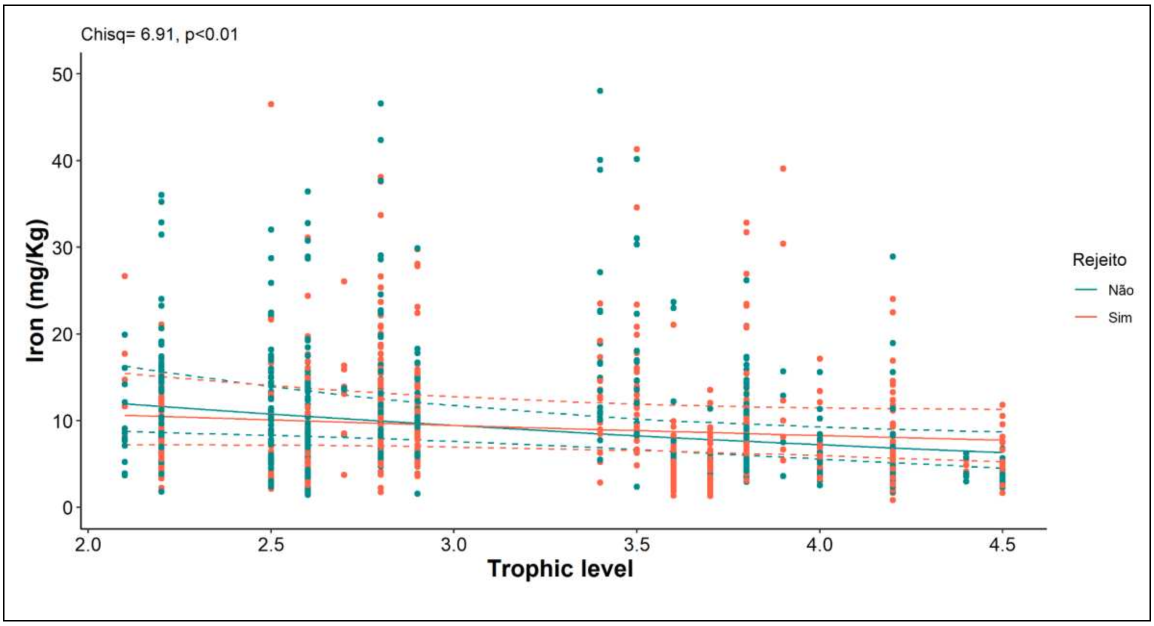


Figure 25: Iron (Fe) concentration in muscle tissue in relation to the trophic level of fish. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). Iron concentration decreased along trophic levels (bioreduction) (χ^2=4.09, p=0.0430) and there was an interaction between the effects of tailings and trophic level, with steeper bioreduction in “unaffected” than in “affected” sites (χ^2=6.91, p= 0.0089).

Hg — Mercury

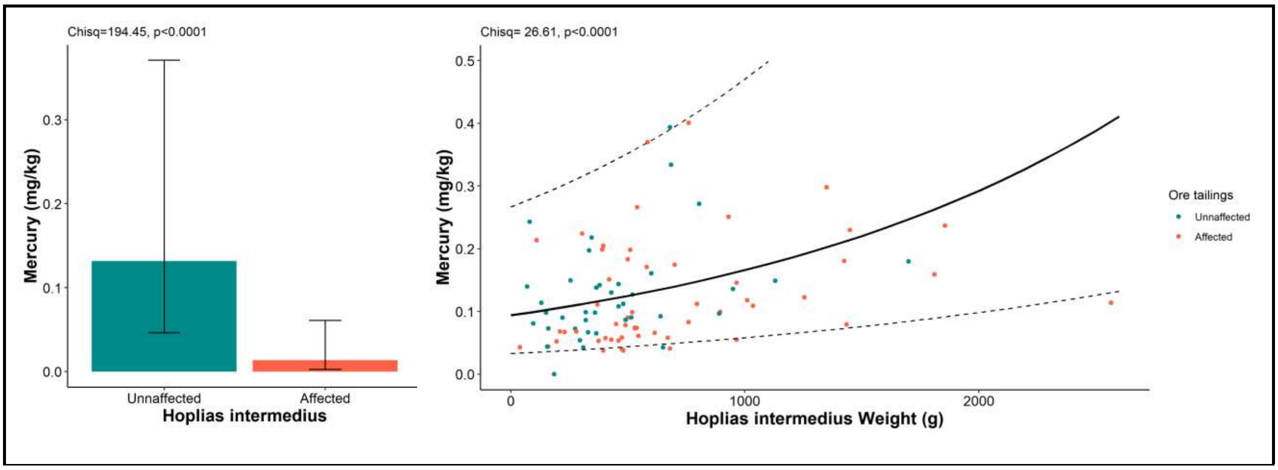


Figure 26: **(A)** Mercury (Hg) concentrations were lower in *Hoplias intermedius* presented lower mercury concentrations in fishes from "affected" sites, compared to fishes from "unaffected" sites, ($\chi 2$=194.45, p=2.13e-6). **(B)** There was bioaccumulation of mercury in *Hoplias intermedius* ($\chi 2$=26.61, p=5.88e-5). Green bar: mercury concentration in fishes from unaffected sites; orange bar: mercury concentration in fishes from affected sites; Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

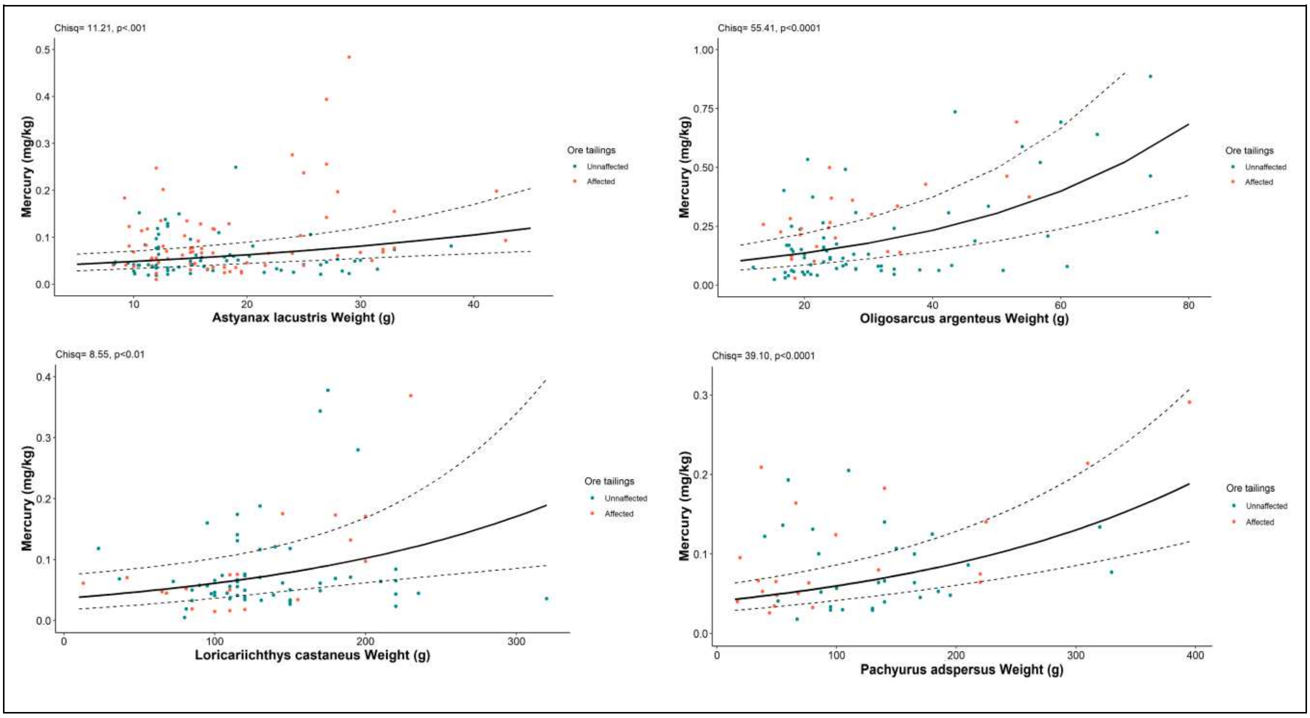


Figure 27: There was bioaccumulation of mercury in four other fish species of the nine evaluated, in both affected and unaffected sites: **(A)** *Astyanax lacustris* ($\chi 2$=11.21, p=0.0009), **(B)** *Oligosarcus argenteus* ($\chi 2$=55.41, p=0.0367), **(C)** *Loricariichthys castaneus* ($\chi 2$=8.55, p=0.0034) and **(D)** *Pachyurus adspersus* ($\chi 2$=39.10, p=8.45e-8). Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

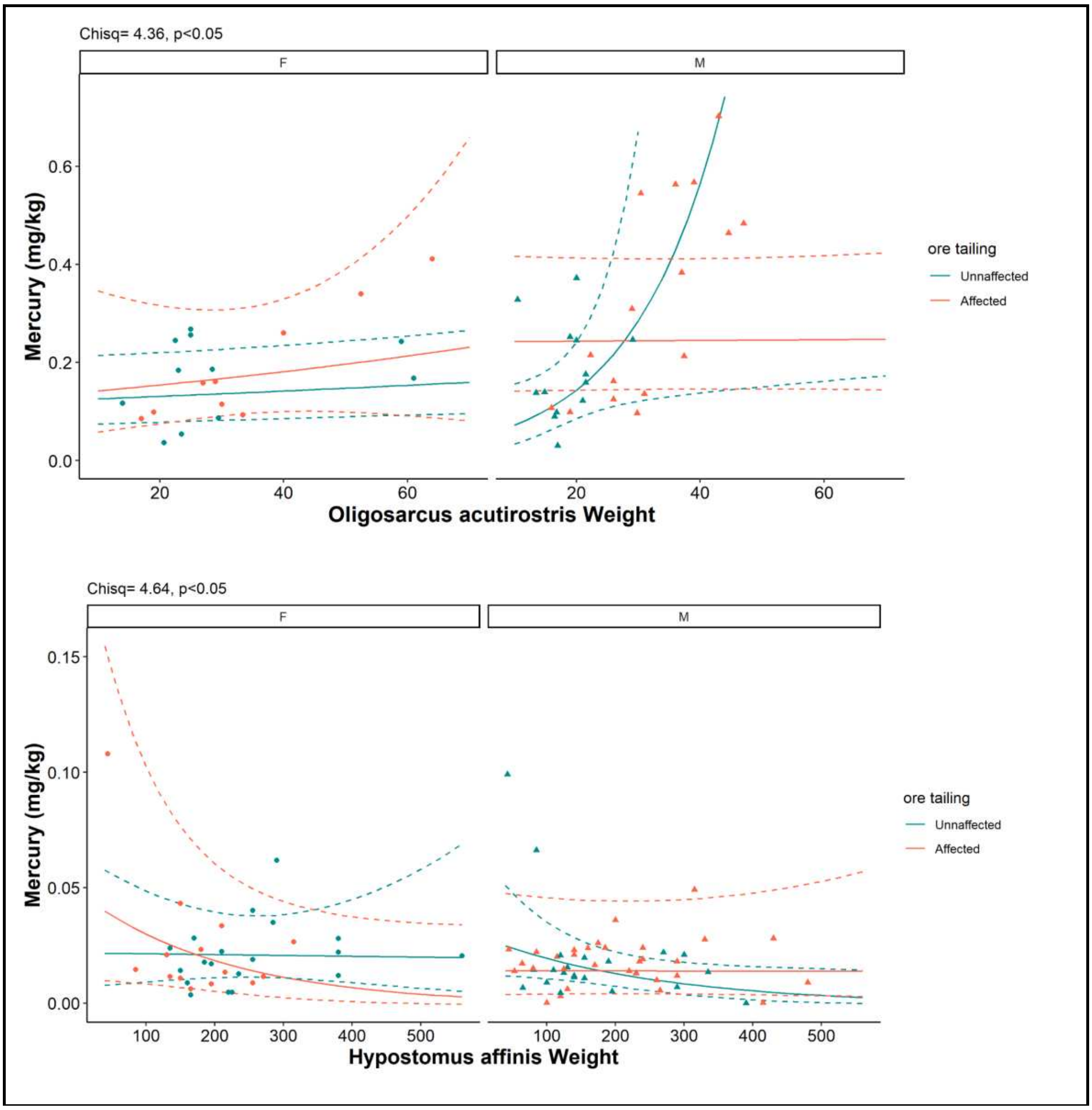


Figure 28: **(A)** *Oligosarcus acutirostris* (χ^2=4.36, p=0.0367) and **(B)** *Hypostomus affinis* (χ^2=4.64, p=0.0038), showing an interaction between the effects of ore tailings, fish sex and fish weight indicating that the bioaccumulation processes differed between male and female fishes, and was also influenced by the ore tailings. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM). We did not represent points that were too distant (one point at B, with very high Hg value), so as to maximize the view of the adjusted curves, but these points were not outliers, as far as their deletion did not change the results qualitatively, and thus these points were maintained in the final adjusted model.

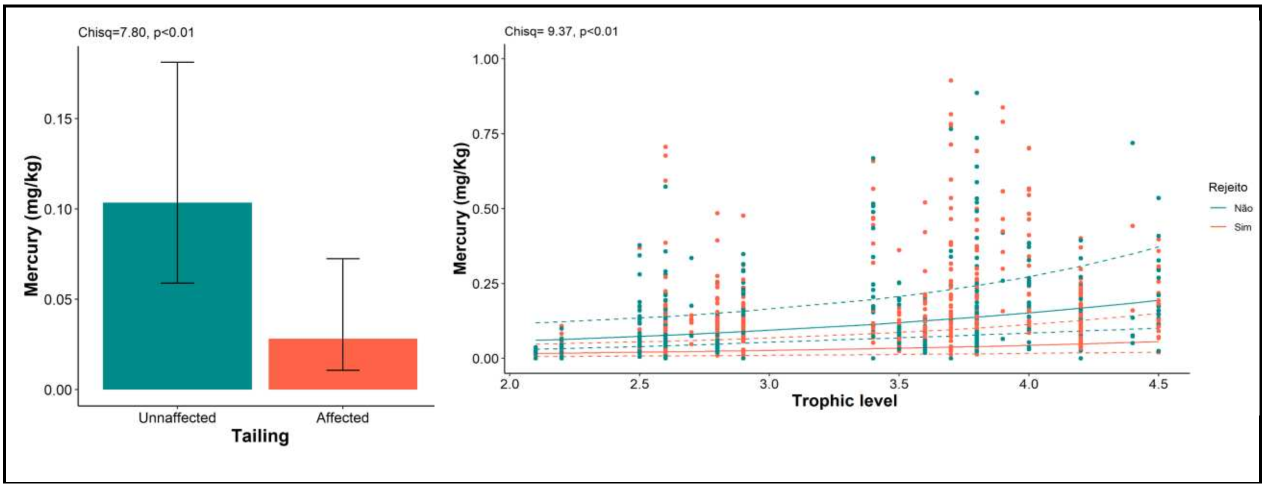


Figure 29: (A) Concentration of Mercury (Hg) is lower in fishes from "affected", compared to "unaffected" sites (χ^2=7.80, p=0.0061). Green bar: fishes in unaffected sites; orange bar: fishes in affected sites. **(B)** Mercury (Hg) biomagnification, increasing its concentration along trophic levels in both "affected" and "unaffected" sites (χ^2=9.37, p= 0.0021).

Mn — Manganese

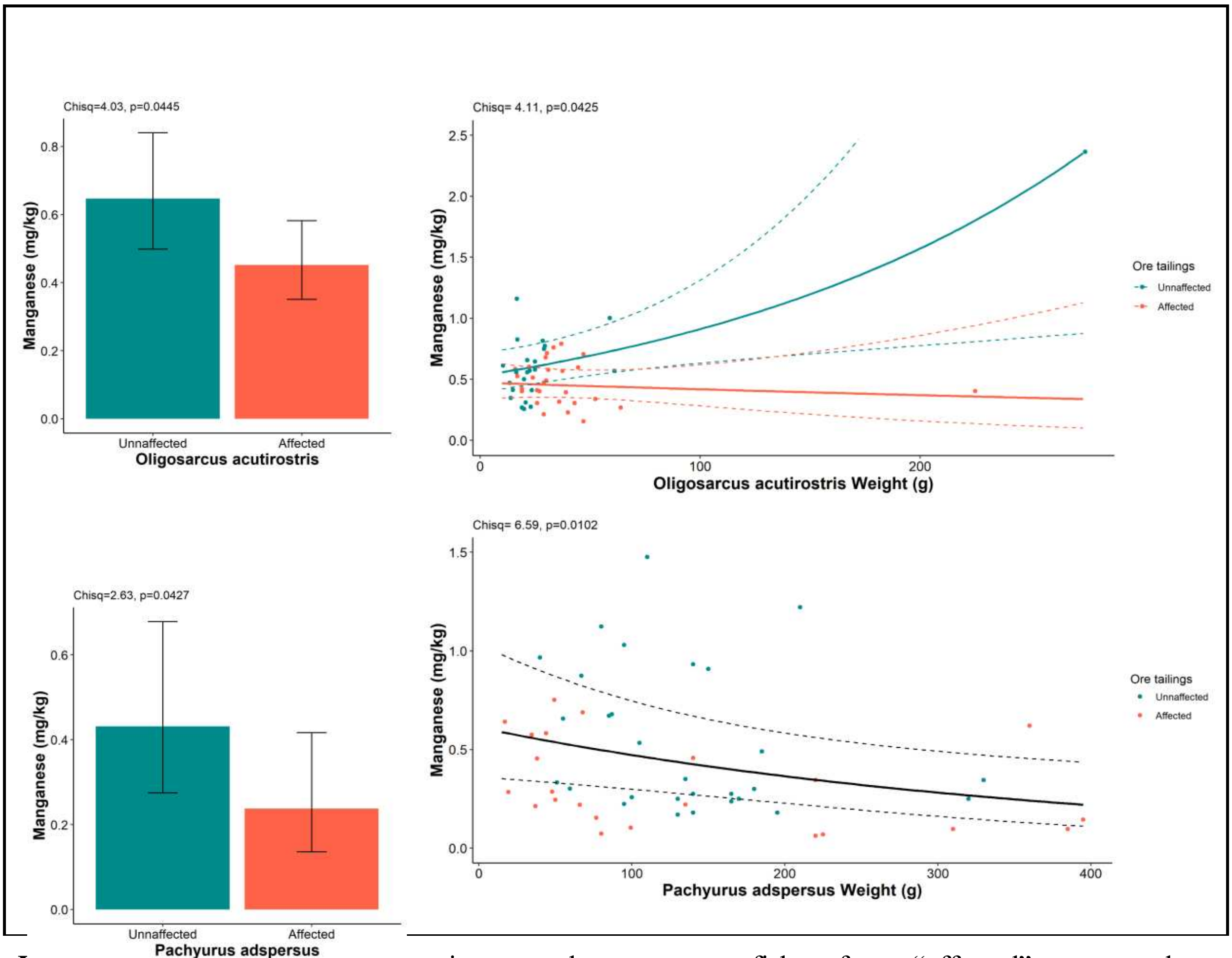


Figure 30: Manganese concentration was lower among fishes from “affected”, compared to “unaffected” sites, in two species: **(A)** *Oligosarcus acutirostris* (χ^2=4.03, p=0.0445) and **(C)** *Pachyurus adspersus* (χ^2=2.63, p=0.0427). **(B)** *Oligosarcus acutirostris* there was an interaction between the effects of fish weight and ore tailings (χ^2=4.11, p=0.0425), indicating that the presence of ore tailings affected the bioaccumulation processes in *O. acutirostris*. **(D)** Biodilution of manganese in *Pachyurus adspersus* (χ^2=6.59, p=0.0083). Green bar: manganese concentration in fishes from unaffected sites; orange bar: manganese concentration in fishes from affected sites; Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

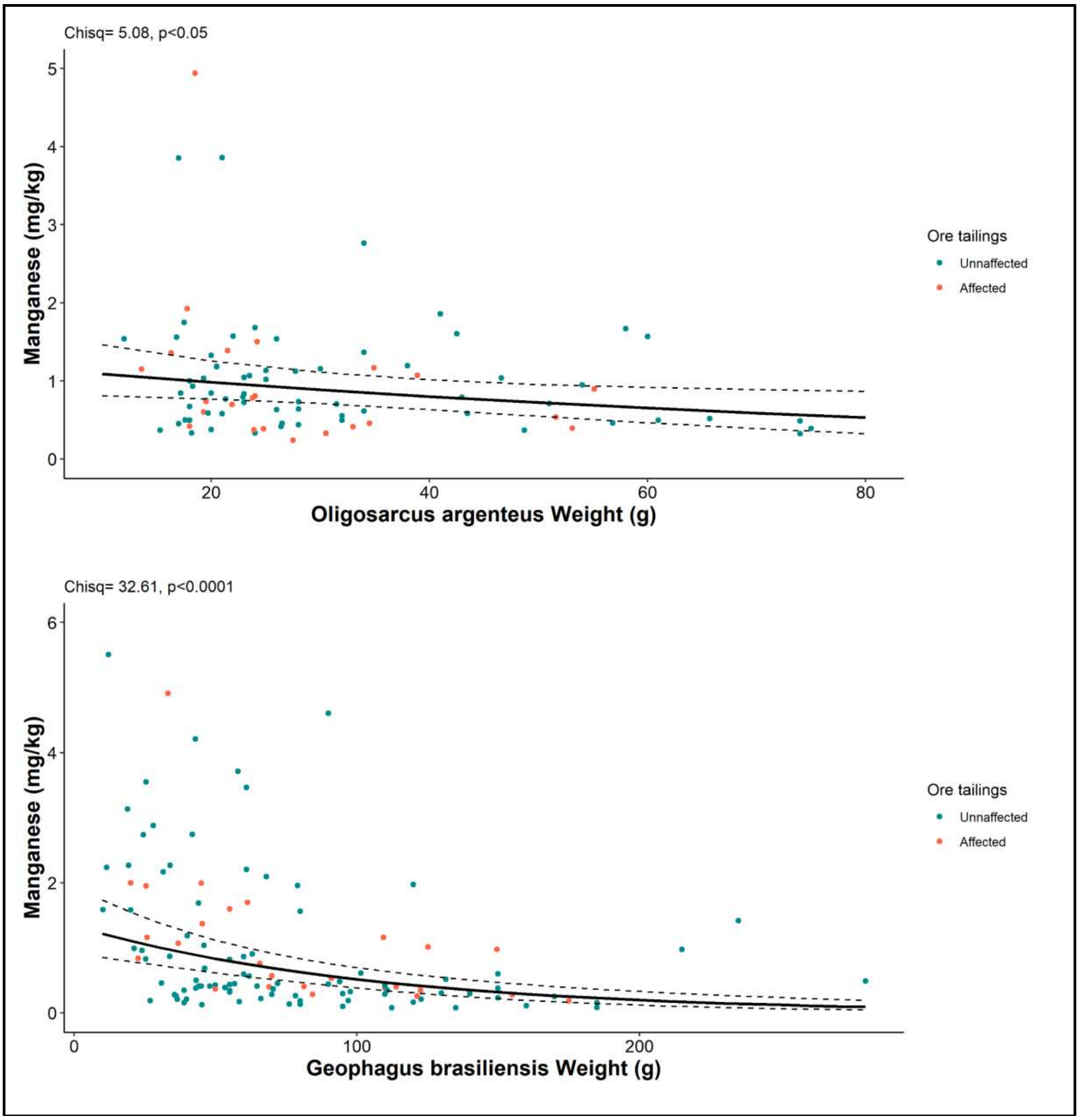


Figure 31: **(A)** Biodilution of manganese in *Oligosarcus argenteus* (χ^2=5.08, p=0.0241) and **(B)** *Geophagus brasiliensis* (χ^2=32.61, p=8.91e^{-8}) muscle tissue, in both affected and unaffected sites. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

Ni — Nickel

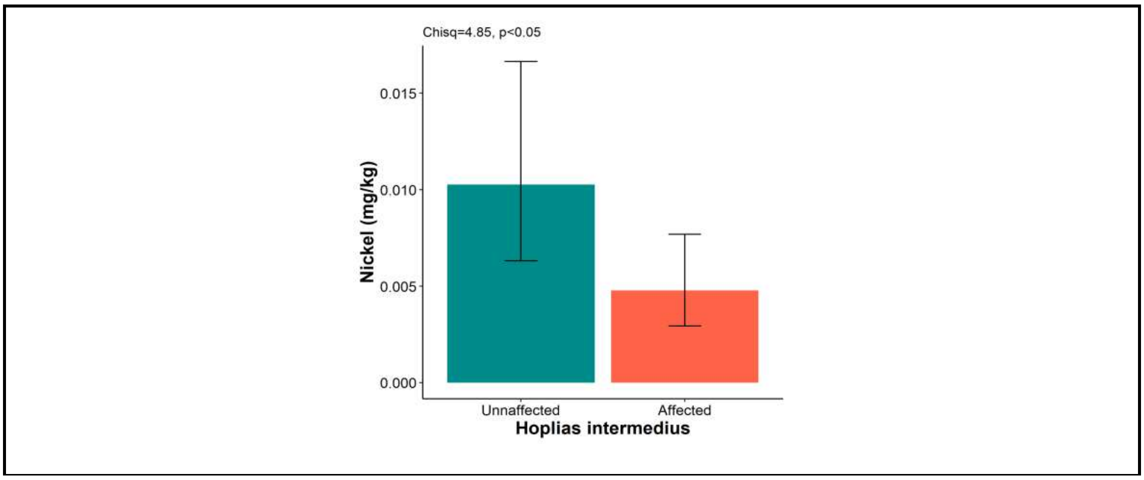


Figure 32: *Hoplias intermedius* had lower nickel values among fishes from "affected", compared to "unaffected" sites (χ^2=4.85, p=0.0275). Green bar: manganese concentration in fishes from unaffected sites; orange bar: manganese concentration in fishes from affected sites.

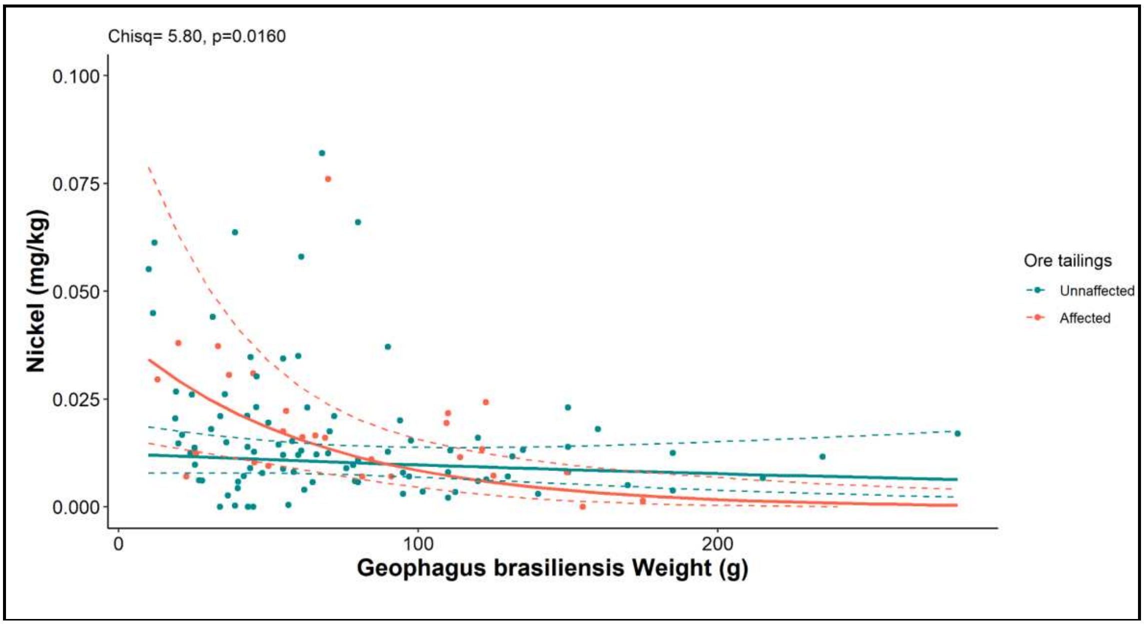


Figure 33: Concentration of nickel (Ni) in *Geophagus brasiliensi*s muscle tissue in relation to weight (g). An interaction between the effects of fish weight and ore tailings in *G. brasiliensis* (χ^2=5.80, p=0.0160). *Geophagus brasiliensis* there was a decrease in concentrations of nickel with fish weight (biodilution) in both "affected" and "unaffected" sites, but the presence of the ore tailings increased biodilution. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

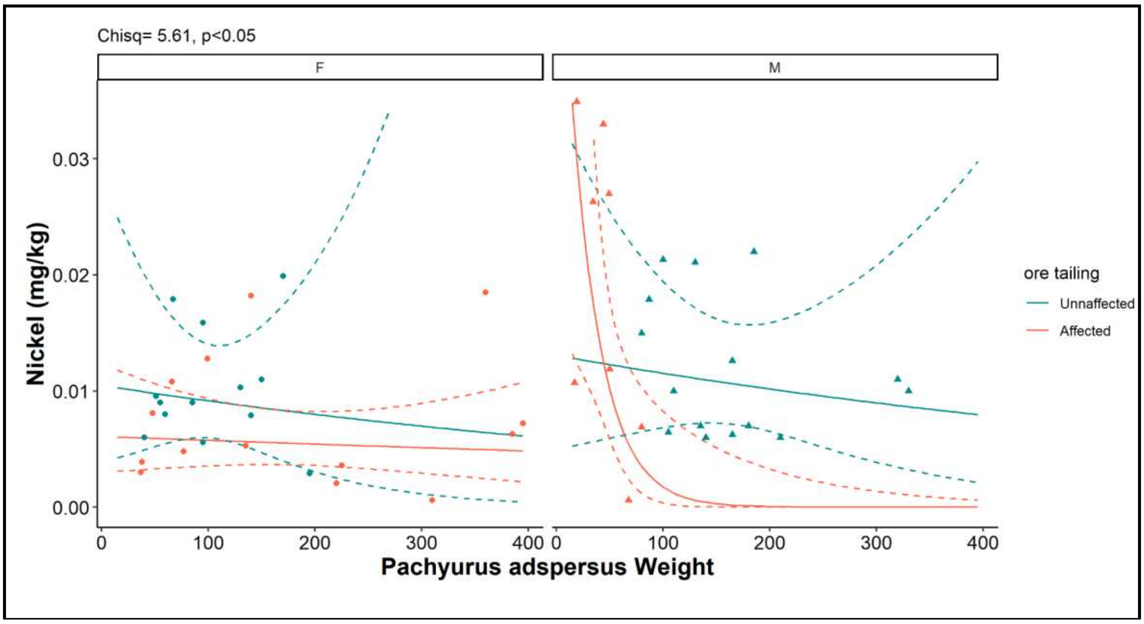


Figure 34: Concentration of nickel (Ni) in *Pachyurus adspersus* muscle tissue. *P. adspersus* showed interaction between the effects of ore tailings, weight and sex, indicating differences in the effects of the ore tailings on the biodilution processes in males in from "affected" sites, were biodilution of nickel was steeper (χ^2=5.61, p=0.0178). Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

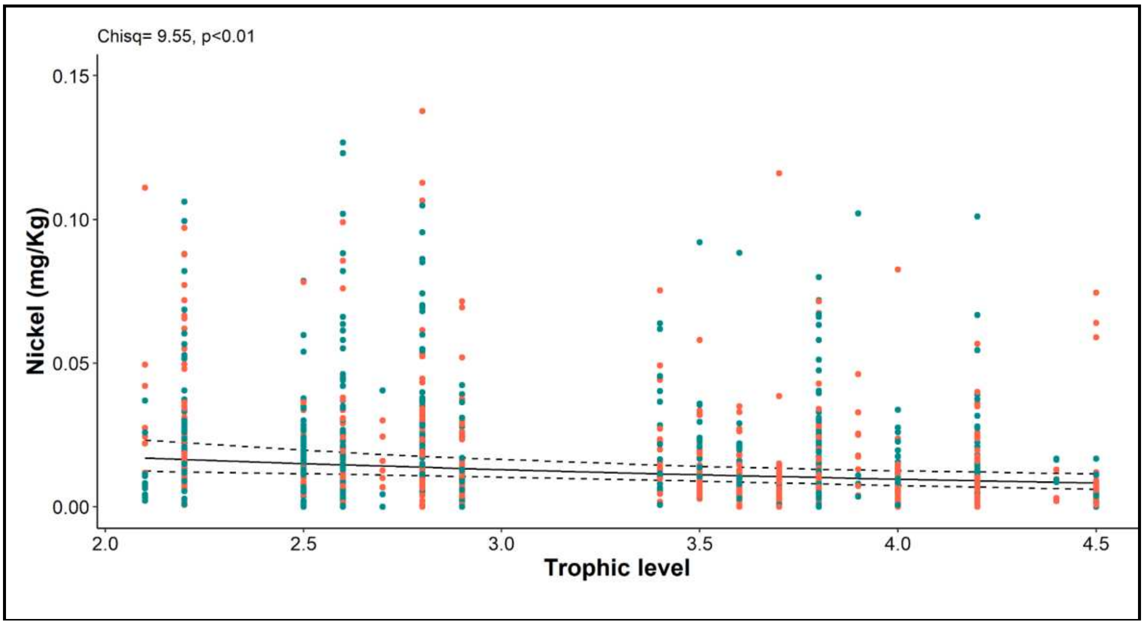


Figura 35: Nickel (Ni) concentration in muscle tissue in relation to the trophic level of fish. Concentration of nickel decreased along trophic levels (bioreduction), with no effects of the ore tailings on nickel concentration (χ^2=9.55, p=0.00199). Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

Pb — Lead

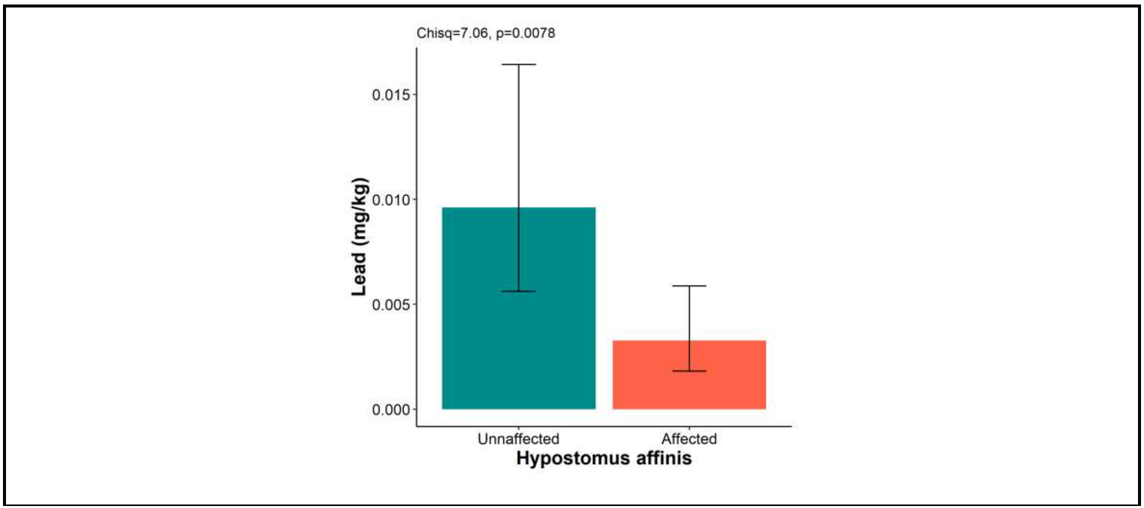


Figure 36: *Hypostomus affinis* had lower lead (Pb) values among fishes from "affected", compared to "unaffected" sites (χ^2=4.85, p=0.0275). Green bar: manganese concentration in fishes from unaffected sites; orange bar: manganese concentration in fishes from affected sites.

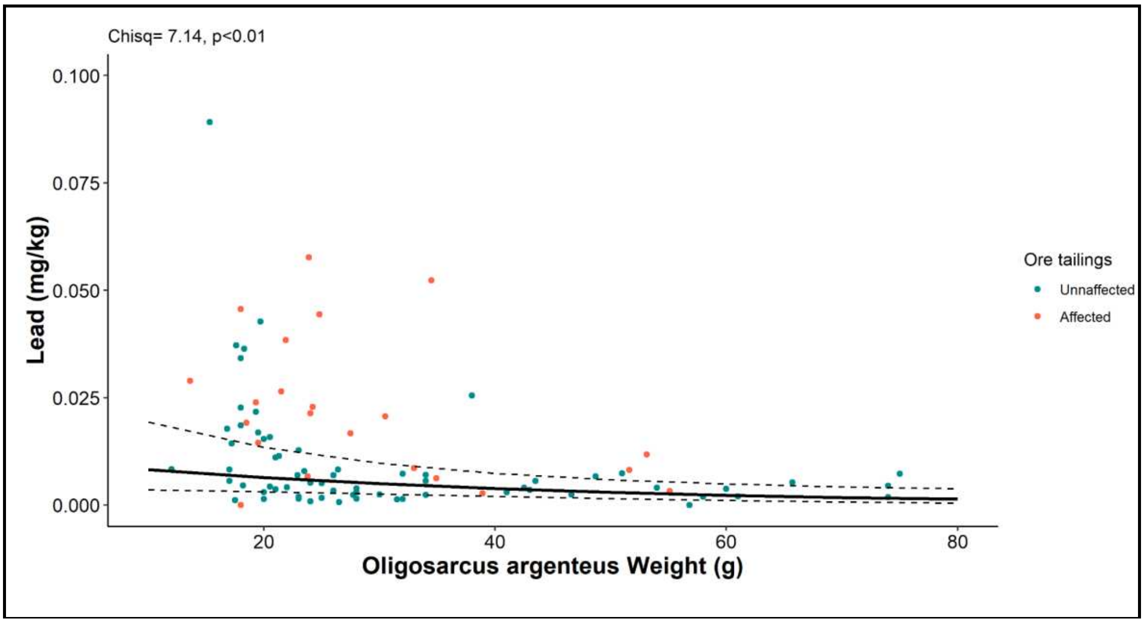


Figura 37: Concentration of lead (Pb) in *Oligosarcus argenteus* muscle tissue. We detected biodilution of lead in *O. argenteus* (χ^2=7.14, p=0.0075), with no effect of ore tailings. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

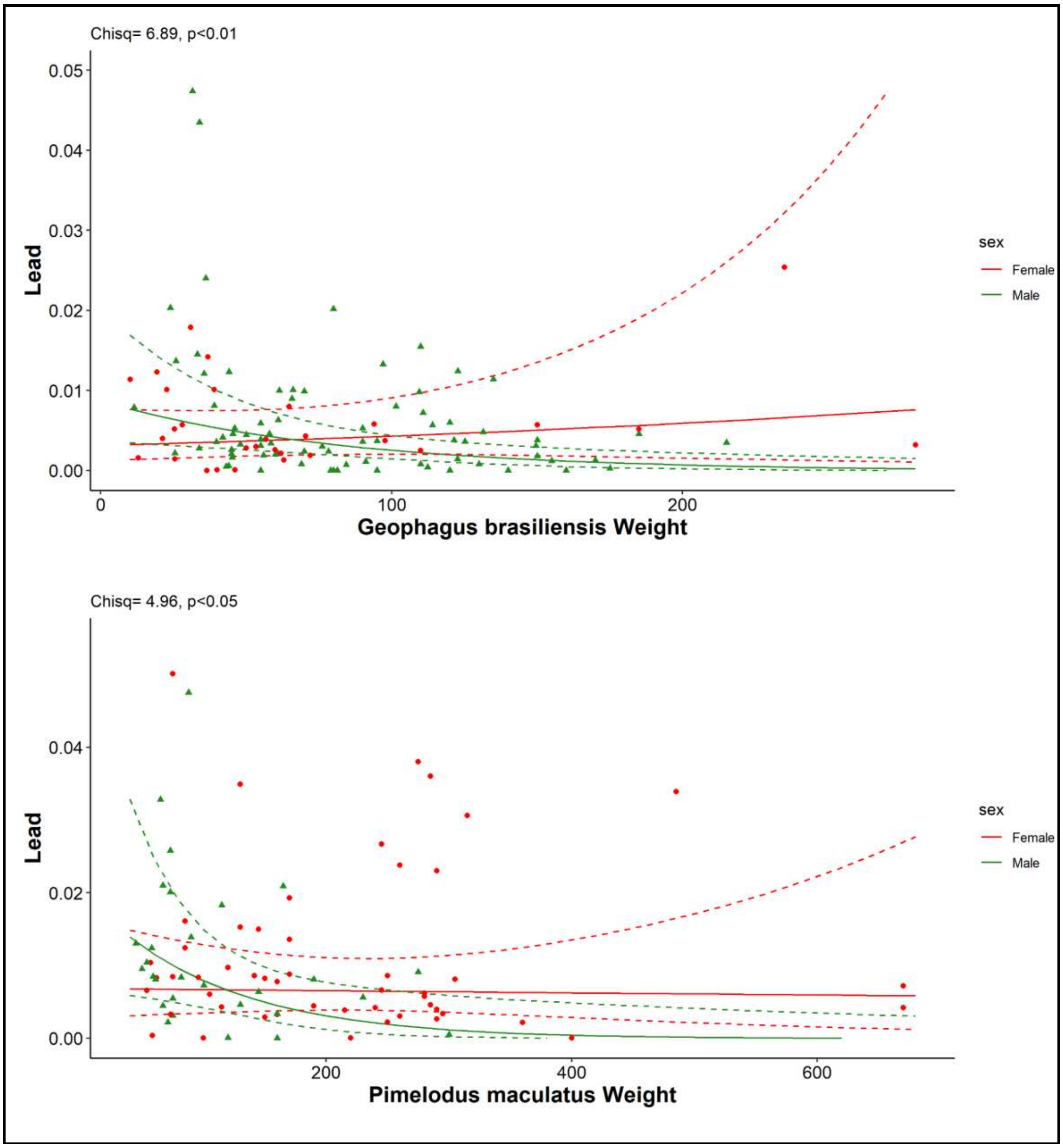


Figure 38: Bioaccumulation processes of lead (Pb). Interaction between the effects of fish weight and fish sex in **(A)** *Geophagus brasiliensis* ($\chi 2$=6.89, p=0.0086) and in **(B)** *Pimelodus maculatus* ($\chi 2$=4.96, p=0.0258, Figure 38-B). In both species, female fishes bioaccumulated lead, while male fishes biodiluted lead. Green dots: Male fishes; red dots: Female fishes; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

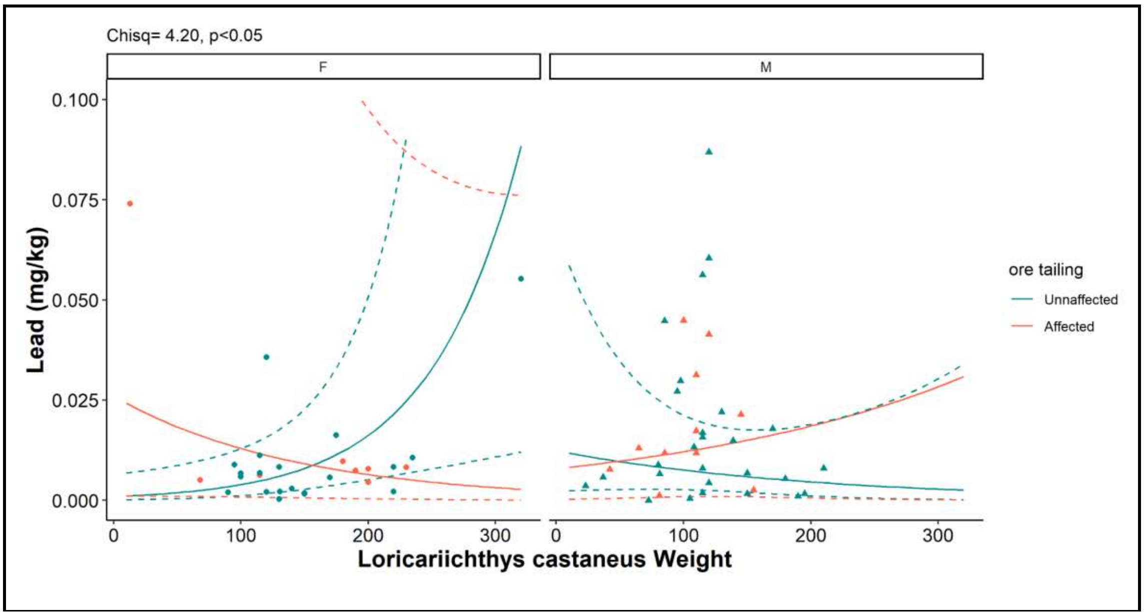


Figure 39: Concentration of nickel (Ni) in *Loricariichthys castaneus* muscle tissue. *L. castaneus* showed interaction between the efects of ore tailings, fish sex and fish weight (χ^2=4.20, p=0.0402): while females biodiluted lead in “affected” sites and bioaccumulated it in “unaffected” sites, males presented the opposite pattern, biodiluting in “unaffectd” and bioaccumulating in “affected” sites. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

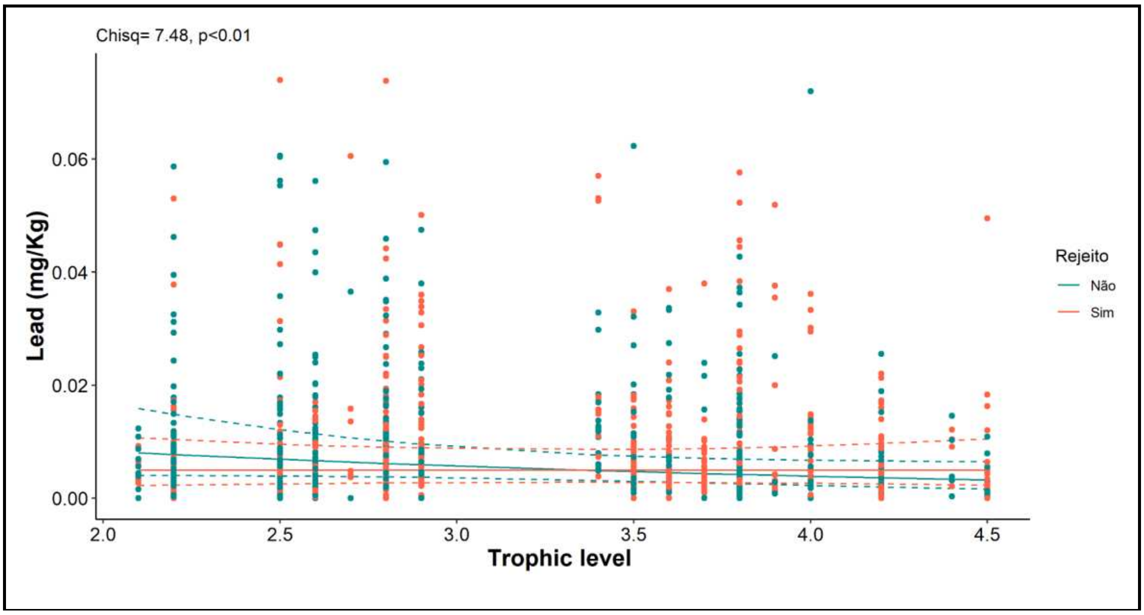


Figure 40: Lead (Pb) concentration did not increase along trophic levels, discarding biomagnification of lead. Was detected an interaction between the effects of ore tailings and trophic level, with a decrease in lead concentrations (bioreduction) in the absence of the ore tailings and apparently no effect of trophic level in the presence of the ore tailings (χ^2=7.48, p= 0.0062). Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

Zn — Zinc

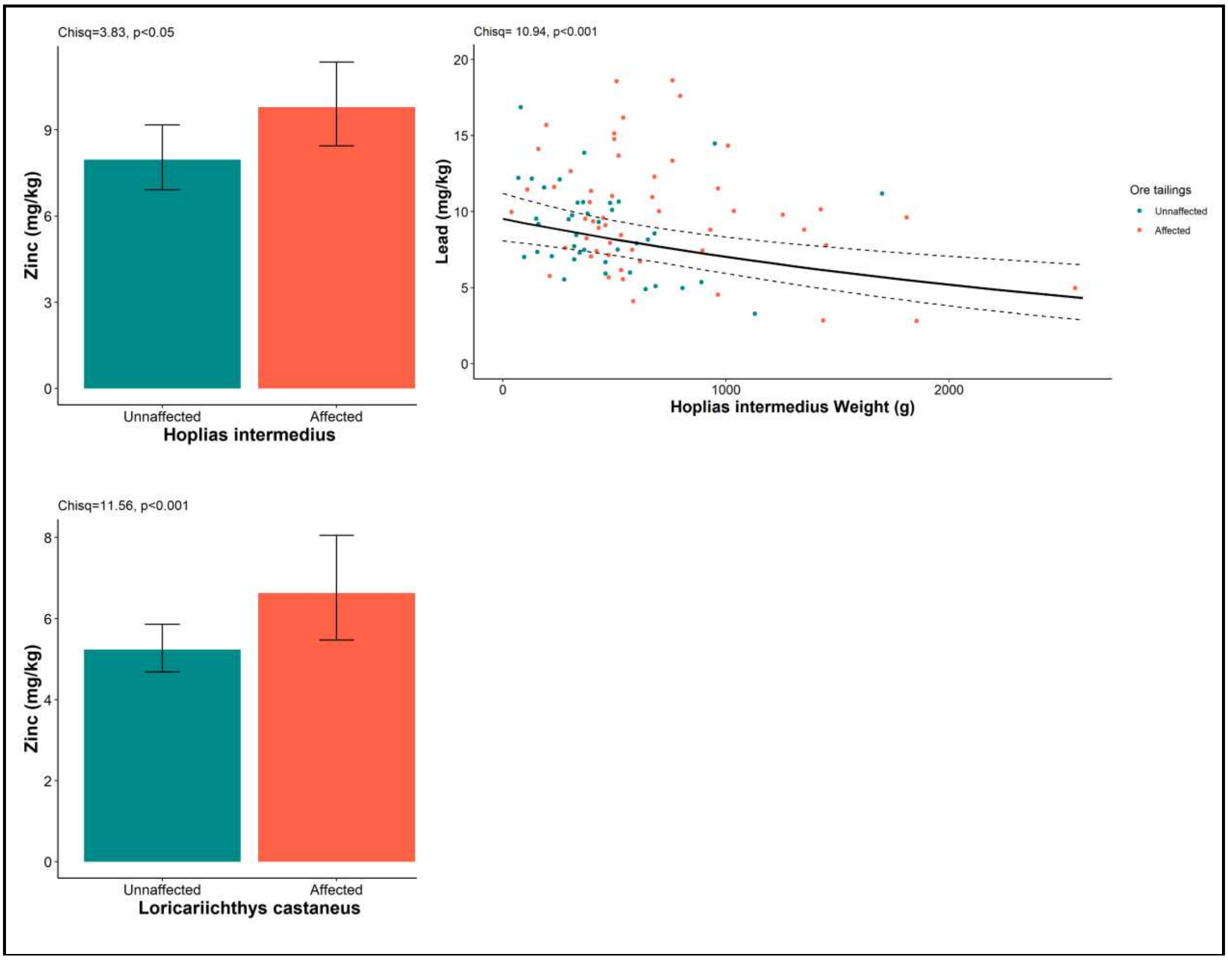


Figure 41: Zinc (Zn) concentration was higher among fishes from "affected", compared to "unaffected" sites, in two species: **(A)** *Hoplias intermedius* (χ^2=3.83, p=0.0455) and **(C)** *Loricariichthys castaneus* (χ^2=11.56, p=0.0008). **(B)** Biodilution of manganese in *Hoplias intermedius*, with no interaction with the effects of the ore tailings (χ^2=10.94, p=0.0010). Green bar: zinc concentration in fishes from unaffected sites; orange bar: zinc concentration in fishes from affected sites; Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

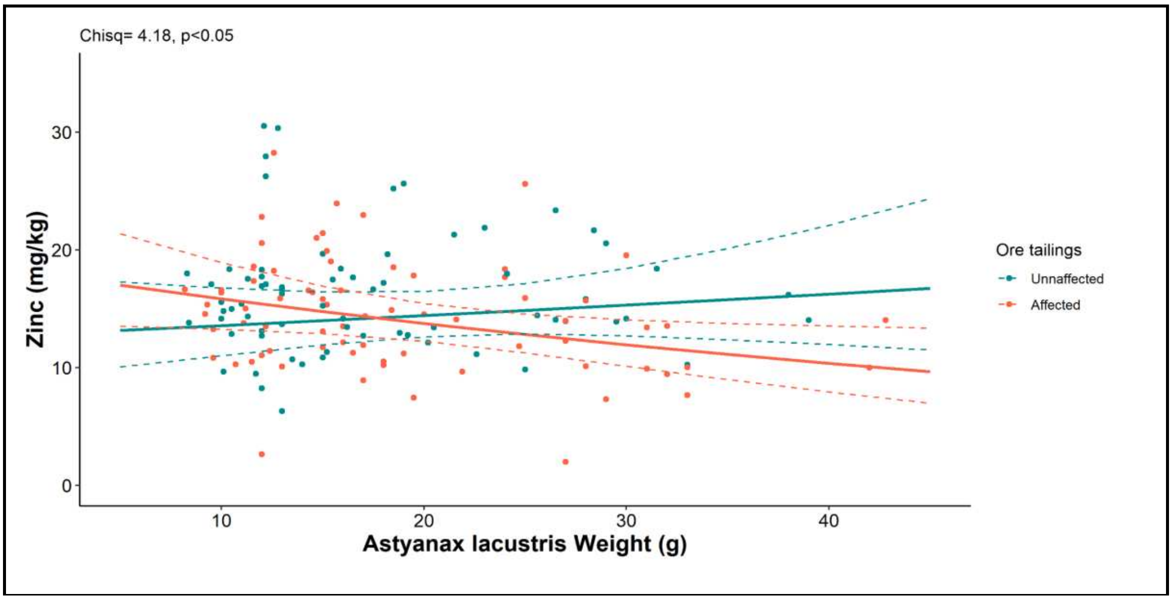


Figure 42: Concentration of zinc (Zn) in *Astyanax lacustris* muscle tissue. While zinc concentration increased with fish weight (bioaccumulation) in unaffected sites, affected sites this process was reversed (biodilution) (χ^2=4.18, p=0.0408). Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

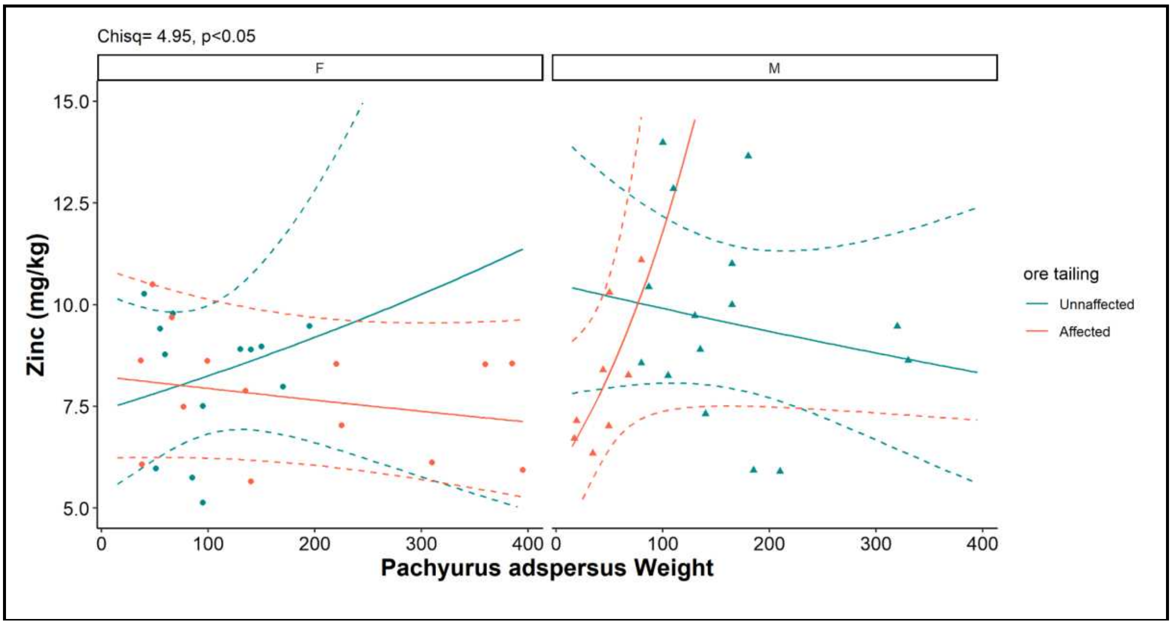


Figure 43: Concentration of zinc in *Pachyurus adspersus* muscle tissue. There was an interaction between the effects of ore tailings, fish sex and fish weight in *Pachyurus adspersus* (χ2=4.95, p=0.0260): while females biodiluted zinc in affected sites and bioaccumulated it in unaffected sites, males presented the opposite pattern, biodiluting zinc in unaffectd and bioaccumulating it in affected sites. Green dots: fishes in unaffected sites; orange dots: fishes in affected sites; curves represent 95% confidence intervals, for the minimum adequate model (GLMM).

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CONSIDERAÇÕES FINAIS

- O rejeito alterou características físico químicas da água como sua condutividade, alterando a bioacumulação de As, Al, Cu e Zn em peixes, o que justifica um monitoramento da concentração destes metais nos peixes, para a segurança alimentar das populações ribeirinhas, e para garantir a atividade pesqueira na bacia.

- Devido a suas características ecológicas, algumas espécies de peixes são mais sensíveis à bioacumulação de elementos químicos, como *Hoplias intermedius* e *Loricariichthys castaneus*, e alguns elementos são mais biodisponíveis no ambiente aquático, como As e Hg. Estas espécies e estes elementos devem ser alvo de monitoramento.

- Encontramos biomagnificação de Hg, em toda a bacia, independente da presença do rejeito. Porém, o rejeito modificou os processos de biomagnificação, reforçando as evidências de interação físico-química do rejeito de minério com a biodisponibilidade de metais e seu acúmulo na biota aquática.

- Os peixes do rio Gualaxo do Norte foram os únicos com concentração média de mercúrio superior ao estabelecido pela ANVISA para consumo humano.

- Em projetos de continuidade (experimentos em andamento) iremos avaliar, em laboratório, o efeito do rejeito e da água do Rio Doce no valor adaptativo dos peixes, utilizando métricas de crescimento e fisiologia, testando tanto os efeitos do rejeito quanto os efeitos sinérgicos da química da água do rio Doce na biodisponibilidade e bioacumulação de metais, a fim de fornecer uma compreensão sólida e segura dos efeitos do rompimento da barragem da SAMARCO, na biota e na segurança alimentar humana.