UNIVERSIDADE FEDERAL DE MINAS GERAIS
Engineering School
Graduate Program in Sanitation, Environment and Water Resources

Gisele Alves Miranda

ECOTOXICOLOGICAL RISK ASSESSMENT OF METALS AND METALLOIDS IN THE PARAOPEBA RIVER REGION AFFECTED BY THE DAM COLLAPSE IN BRUMADINHO/MG BASED ON NOVEL PNEC VALUES ESTABLISHED BY SSD APPROACH

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Dissertation presented to the Graduate Program in Sanitation, Environment and Water Resources of the Universidade Federal de Minas Gerais, as a partial requirement for obtaining the title of Master in Sanitation, Environment and Water Resources.

Research Field: Environmental Sciences

Research Line: Pollution characterization, prevention and control

Advisor: Dr. Maria Clara Vieira Martins Starling

Co-advisor: Dr. Camila Costa de Amorim Amaral

Belo Horizonte
2023

Miranda, Gisele Alves.
M672e Ecotoxicological risk assessment of metals and metalloids in the Paraopeba river region affected by the dam collapse in Brumadinho/MG based on novel PNEC values established by SSD approach [recurso eletrônico] / Gisele Alves Miranda.- 2023.
1 recurso online (137 f. : il., color.): pdf.

Orientadora: Maria Clara Vieira Martins Starling.
Coorientadora: Camila Costa de Amorim Amaral.

Mestrado (dissertação) - Universidade Federal de Minas Gerais, Escola de Engenharia.

Apêndices: f. 125-137.

Bibliografia: f. 108-124.
Exigências do sistema: Adobe Acrobat Reader.

1. Engenharia sanitária - Teses. 2. Meio ambiente - Teses. 3. Ferro - Teses. 4. Biodiversidade - Teses. 5. Barragem - Teses. 6. Resíduos - Teses. 7. Recursos hídricos - Teses. 8. Água - Qualidade - Teses. 9. Mineração - Teses. I. Starling, Maria Clara Vieira Martins. II. Amaral, Camila Costa de Amorim. III. Universidade Federal de Minas Gerais, Escola de Engenharia. IV. Título.

CDU: 628(043)

Ficha catalográfica elaborada pela bibliotecária Ângela Cristina Silva CRB/6 2361
Biblioteca Prof. Mário Werneck, Escola de Engenharia da UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS
[ESCOLA DE ENGENHARIA]
COLEGIADO DO CURSO DE GRADUAÇÃO / PÓS-GRADUAÇÃO EM [SANEAMENTO, MEIO AMBIENTE E RECURSOS HÍDRICOS]

FOLHA DE APROVAÇÃO

"Ecotoxicological Risk Assessment Of Metals And Metalloids In The Paraopeba River Region Affected By The Dam Collapse In Brumadinho/mg Based On Novel Pnec Values Established By Ssd Approach"

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Coordenadora Orientadora

Belo Horizonte, 31 de março de 2023.

Documento assinado eletronicamente por **Camila Costa de Amorim Amaral**, **Professora do Magistério Superior**, em 31/03/2023, às 15:51, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Cristiano Christofaro Matosinhos**, **Usuário Externo**, em 31/03/2023, às 15:52, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Referência: Processo nº 23072.218284/2023-45 SEI nº 2187455

AGRADECIMENTOS

Em primeiro lugar, agradeço a Deus, por me dar força, saúde e perseverança todos os dias. Por me permitir alcançar mais esse sonho, por me guiar e proteger meus passos, levando-me aos melhores caminhos.

À minha família. Aos meus pais, Graça e Orlando, por serem meu porto seguro. Por sempre me incentivarem e apoiarem minhas decisões, com amor, carinho e paciência. À minha irmã e amiga, Sílvia, pela parceria, amizade e pelas conversas reconfortantes. Também por tornar a vida mais leve, com seu jeito humilde, calmo e extrovertido.

Ao meu marido e melhor amigo, Fábio. Pelo amor, compreensão, paciência, dedicação e auxílio. Por sempre dar força aos meus sonhos, por me ouvir, apoiar e aconselhar. Por me acalmar e me permitir enxergar o lado positivo em tudo o que acontece em nossas vidas. Também a sua família, pela força, torcida e amizade.

À minha orientadora, professora Maria Clara, e à minha coorientadora, professora Camila, grandes exemplos profissionais, pelas oportunidades, conhecimentos transmitidos e disponibilidade em contribuir com meu crescimento acadêmico, pessoal e profissional. Pela confiança e reconhecimento do meu trabalho.

Aos meus amigos, em especial, Flávia, Jéssica, Meiryanne e Gleuber, que, mesmo de longe, estão sempre presentes.

À Marcela e ao professor Rhaul, que tanto auxiliaram para que esse trabalho pudesse ser concluído.

Aos meus primeiros orientadores, em especial às professoras Adelaide, Míriam e Laura, com quem pude iniciar a vida acadêmica e adquirir conhecimentos tão importantes para a realização desse trabalho.

Aos membros da banca examinadora, Wanderlene, Cristiano e Beatriz, que gentilmente aceitaram o convite para a avaliação deste trabalho e que contribuíram para sua versão final.

À Agência Nacional de Águas e Saneamento Básico (ANA), pela disponibilização dos dados.

Aos professores do Programa de Pós-Graduação em Saneamento, Meio Ambiente e Recursos Hídricos (SMARH), pelos conhecimentos transmitidos e incentivos constantes.

A todos os amigos e colegas do SMARH, mesmo aqueles que não conheci pessoalmente, por tornarem o ensino remoto, em tempos de pandemia, mais agradável.

À secretaria do SMARH, em especial ao Júlio e ao Lucas, pela dedicação, prontidão e auxílio com as questões burocráticas.

À UFMG e à Escola de Engenharia pelo amparo concedido para o desenvolvimento da pesquisa.

E a todos os amigos e familiares que contribuíram de alguma forma para a realização deste trabalho e para a conclusão de mais essa etapa.

ABSTRACT

The failure of the B1 dam in Brumadinho, on January 25th, 2019, released about 10 Mm³ of tailings to the environment, of which a parcel reached the Paraopeba River. Several impacts resulted from this collapse, such as toxic effects to the aquatic biota. Although ecotoxicological assays are essential to assess the ecological status of aquatic ecosystems, they demand time and resources, which can make long-term monitoring more challenging. In contrast, ecotoxicological risk assessment methods, such as the Species Sensitivity Distribution (SSD), aligned to the calculation of risk quotients, allow for a robust evaluation of the ecological risk. Thus, the general goal of this study was to define novel Predictec No Effec Concentration (PNEC) values for metals and metalloids by SSD curves for different trophic levels and to perform a spatial and temporal evaluation of ecotoxicological risks associated to these contaminants in the surface water of the Paraopeba River region affected by the B1 dam collapse. In a first phase of the study, acute and chronic SSD curves were constructed for three trophic levels – producers, primary and secondary consumers – for 14 metals, to determine the HC_5 (hazardous concentration for 5% of species) and the PNEC values, which were compared to legal limits established worldwide. For acute and chronic effects, respectively, 32 and 78% of the estimated PNEC values were below the Brazilian legislation limits, which were defined more than 18 years ago, indicating that they might not be protective to the aquatic biota. Ag was the most critical element, as all PNEC values were under current legal limits. In a second stage, ecotoxicological risks were estimated based on the most restrictive PNEC values calculated for each compound and on water quality data from the Paraopeba River. The evaluation of temporal and spatial evolution of risks along 25 monitoring points indicated that Fe represented the higher risk in the Paraopeba River, followed by Al. During the studied period, regions located from 400 m upstream to 68,4 km downstream the B1 dam collapse point were the most impacted, thus requiring greater efforts for watershed restoration and aquatic life protection. Results obtained in this study may be an important guide to the definition of effective environmental standards for aquatic life protection in Brazil and in Minas Gerais, and also a tool for watershed management decision-making in areas affected by different anthropic activities or pollution events related to metal contamination.

Keywords: Ecological Risk Assessment. Surface Water Quality. Iron Ore Mining. Species Sensitivity Distribution (SSD). Biodiversity.

RESUMO

O rompimento da Barragem B1 em Brumadinho, em 25 de janeiro de 2019, liberou cerca de 10 Mm^3 de rejeitos para o meio ambiente, parte dos quais alcançou o Rio Paraopeba. Diversos impactos resultaram desse colapso, como efeitos tóxicos à biota aquática. Embora ensaios ecotoxicológicos sejam essenciais para avaliar o estado ecológico dos ecossistemas aquáticos, eles demandam tempo e recursos, o que pode tornar o monitoramento de longo prazo mais desafiador. Por outro lado, métodos de avaliação de risco ecotoxicológico, como a Distribuição de Sensibilidade de Espécies (SSD), alinhados ao cálculo de quocientes de risco, permitem uma avaliação robusta do risco ecológico. Assim, o objetivo geral deste estudo foi definir novos valores de PNEC (Concentração Prevista de Efeito Não Observado) para metais e metaloides, por meio de curvas SSD construídas para diferentes níveis tróficos e realizar avaliação espacial e temporal dos riscos ecotoxicológicos associados a esses contaminantes nas águas da região do Rio Paraopeba afetada pelo rompimento da Barragem B1. Em uma primeira etapa do estudo, foram construídas curvas SSD para efeitos agudos e crônicos para três níveis tróficos – produtores, consumidores primários e secundários – para 14 metais, de forma a se determinar os valores HC_5 (concentração de perigo a 5% das espécies) e de PNEC, que foram comparados aos limites legais estabelecidos em diversas localidades do mundo. Para efeitos agudos e crônicos, respectivamente, 32 e 78% dos valores estimados dos PNECs ficaram abaixo dos limites da legislação brasileira, que foram estabelecidos há mais de 18 anos, indicando que esses limites podem não ser protetivos à biota aquática. Ag foi o metal mais crítico, uma vez que todos os valores de PNEC ficaram abaixo dos limites legais. Em uma segunda etapa, os riscos ecotoxicológicos foram estimados com base nos valores de PNEC mais restritivos calculados para cada composto e nos dados de qualidade da água do Rio Paraopeba. A avaliação da evolução temporal e espacial dos riscos ao longo de 25 pontos de monitoramento indicou que o Fe apresentou os maiores riscos no Rio Paraopeba, seguido pelo Al. Durante o período estudado, as regiões localizadas entre 400 m a montante e 68,4 km a jusante do ponto de rompimento da barragem B1 foram as mais impactadas, demandando, portanto, maiores esforços para recuperação da bacia hidrográfica e proteção da vida aquática. Os resultados obtidos neste estudo podem ser um importante guia para a definição de padrões ambientais efetivos para proteção da vida aquática no Brasil e em Minas Gerais, além de servir como uma ferramenta para a tomada de decisões no âmbito da gestão de bacias hidrográficas em áreas afetadas por diferentes atividades antrópicas ou eventos de poluição relacionados à contaminação por metais.

Palavras-chave: Avaliação de Risco Ecológico. Qualidade da Água Superficial. Mineração de Ferro. Distribuição de Sensibilidade de Espécies (SSD). Biodiversidade.

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LIST OF ABBREVIATION AND ACRONYMS

ABNT – Associação Brasileira de Normas Técnicas

AF – Assessment Factor

ANA – Agência Nacional de Águas e Saneamento Básico (National Water and Sanitation Agency)

BioF – Bioavailability Factor

BLM – Biotic Ligand Model

CA – Cluster Analysis

CERH – Conselho Estadual de Recursos Hídricos (Minas Gerais state Water Resources Council)

CONAMA – Conselho Nacional de Meio Ambiente (National Environmental Council)

COPAM – Conselho Estadual de Política Ambiental (Minas Gerais state Environmental Policy Council)

DOC – Dissolved Organic Carbon

EC_{10} – Effect Concentration to 10% of the population

EC_{50} – Effect Concentration to 50% of the population

HC_5 – Hazard Concentration to 5% of evaluated species

HC_{5bio} – Hazard Concentration to 5% of evaluated species, considering the bioavailable fraction

KW – Nonparametric Kruskal-Wallis test

LC_{50} – Median Lethal Concentration

LOEC – Lowest Effect Concentration

LOQ – Limit of Quantification

MABH – Metropolitan Area of Belo Horizonte

MC – Multiple comparison test

MEC – Measured Environmental Concentration

NOEC – No Effect Concentration

PMQAS – Programa de Monitoramento de Qualidade das Águas Superficiais e Sedimentos

PNEC – Predicted No Effect Concentration

RA to RF – Regions A to F

RQ – Risk Quotient

SPM – Suspended Particulate Matter

SS – Suspended Solids

SSD – Species Sensitivity Distribution

WQG – Water Quality Guideline

α – Significance Level

SUMMARY

1 INTRODUCTION

Failure of tailings dams has become more frequent over the years. From a total of 153 events involving dam collapses in the world from 1960 to 2022, 27% happened between 2011 and 2020, showing an average of 4.1 occurrences per year. In comparison with the previous decade (2001-2010), the number of failures doubled. During the two first years of the present decade (2021-2022), the annual average jumped to 5. If this rate remains, about 50 collapses might occur by the end of 2030 (WISE URANIUM PROJECT, 2023).

In the Brazilian context, Minas Gerais (MG) and Pará are the states where mining activity predominates. In the third quarter of 2022, Minas Gerais responded to 39% of the national mineral production which corresponded to R$ 29.7 billion (IBRAM, 2022). On the other hand, seven tailings dams collapsed in Minas Gerais on the last 22 years (WISE URANIUM PROJECT, 2023). Among those, the B1 dam of Vale S.A, in Brumadinho, that failed on January 25th, 2019, and was the worst dam failure worldwide in terms of human life loss as it led to 270 deaths in total (ROTTA *et al.*, 2020; VALE, 2023). Besides loss of human lives, the tailings wave released by the dam also killed domestic and wildlife, compromised water supply, destroyed residences, and working areas, destroyed riparian vegetation and forested areas and altered physicochemical properties of the Paraopeba River water and sediments (CIONEK *et al*., 2019; PARENTE *et al*., 2021; SOARES; OLIVEIRA; GOMES, 2022). Furthermore, the collapse caused social impacts, such as suffering to those who lost relatives, friends and coworkers; were obliged to leave their residences and lost their source of income and reference of safety (VALE, 2022).

Considering the increasing number of dam collapses over the years and their impacts to the environment, society and human health, it is critical to study these events from all these perspectives. The scientific literature has responded to that, as studies in this field became more frequent. Most studies approach the causes related to different collapses (GLOTOV *et al.*, 2018; HU *et al.*, 2021; LABANDA *et al.*, 2021; RICO; BENITO; DÍEZ-HERRERO, 2008; ROTTA *et al.*, 2020) or their impact upon water and sediments quality in the watershed (FERNANDES *et al.*, 2016; KOSSOFF *et al.*, 2014; QUEIROZ *et al.*, 2018; THOMPSON *et al.*, 2020; VERGILIO *et al.*, 2021). However, studies related to impact assessment of mining dams collapses upon aquatic life via ecotoxicological approaches are still scarce (ALMEIDA *et al.*, 2021; SEGURA *et al.*, 2016; WEBER *et al.*, 2020).

Furthermore, most published studies focus on the correlation between dam failure and the evolution of physicochemical parameters (for example: turbidity, suspended solids, metals and metalloids concentration) in water and sediments. However, temporal reduction in concentrations of substances in the water column may not result in habitat recovery nor absence of toxicity to aquatic organisms as some substances may show toxicity even at low concentrations and toxicity effects may be a consequence of additive or synergistic effects.

For instance, the study developed by Vergilio *et al*. (2021) showed that, despite decrease in turbidity, concentrations of suspended particulate matter (SPM) and metals along the Doce River after the Fundão tailings dam collapse in Mariana/MG (2015), water and sediment samples collected along the river were still toxic to aquatic biota even six months after the event.

In this way, it is critical to perform spatiotemporal ecotoxicological evaluation in the watersheds impacted by a dam collapse. Nevertheless, few studies conducted more than two sampling campaigns to evaluate toxic effects associated to water and sediments after the two major dam failures which occurred in MG in the past seven years (CORDEIRO *et al.*, 2019; MENDES *et al.*, 2020). After the B1 dam failure in Brumadinho, several studies detected ecotoxicological effects along the Paraopeba River to different trophic levels, thus indicating a critical need for long-term monitoring of critical regions (PARENTE *et al.*, 2021; TERAMOTO *et al.*, 2021; VERGILIO *et al.*, 2020).

Although water sampling to assess physicochemical and ecotoxicological parameters are essential to assess the quality status of aquatic ecosystems, they demand time and resources, which can make long-term monitoring more challenging. In contrast, indirect risk assessment methodologies, such as those based on Predicted No Effects Concentration (PNEC) values derived from Species Sensitivity Distribution (SSD), allow for temporal and spatial ecotoxicological evaluation, enabling the identification of risk patterns and priority regions for monitoring. This methodology is frequently used for evaluating ecotoxicological risks related to endocrine disrupting compounds (GAO *et al.*, 2014; GUAN *et al.*, 2018; WEE *et al.*, 2019), pesticides (MOURA; SOUZA-SANTOS, 2020; SPILSBURY; WARNE; BACKHAUS, 2020), and metals in freshwater (ALHO *et al.*, 2022; RAZAK *et al.*, 2021), as well as risks related to soil contamination (KIM *et al.*, 2018; KWAK *et al.*, 2020).

So far, only a few studies have reported the application of ecotoxicological risk assessment methods for the evaluation of ecotoxicological impacts of mining tailings dam collapses, and most of them asses risks related to soil and sediments (BUCH *et al.*, 2021; GABRIEL *et al.*, 2020; PAN *et al.*, 2023). Furthermore, studies assessing ecotoxicological risks related to mining tailings dam failures by applying the SSD methodology were not identified in the current literature.

In the context of disasters, such as collapses of tailings dams, initial monitoring actions carried out to assess the quality of water and sediments focus mainly in physicochemical water quality parameters for which there are legal environmental standards. However, legal limits might not be updated, thus lacking to protect aquatic biota and water uses. For example, in Brazil, the Resolution of the Conselho Nacional de Meio Ambiente (CONAMA) n° 357/05 (BRASIL, 2005) defines legal standard values for different classes of freshwater, saline and brackish water bodies. According to Umbuzeiro (2015), most of the Brazilian legal standard values were imported from other countries, such as Canada and United States and were adopted as according to the most restrictive value amongst all water uses corresponding to each class - drinking water, aquatic life protection, water for animal consumption, irrigation, and recreation (UMBUZEIRO; KUMMROW; REI, 2010). For Minas Gerais state, legal limits for water quality are defined by the joint Normative Deliberation n° 08/2022, from Conselho Estadual de Política Ambiental (COPAM) and Conselho Estadual de Recursos Hídricos (CERH) (MINAS GERAIS, 2022). Although the state regulation was published in 2008, its updated version published in November 2022 maintained legal standards for metals and metalloids which were based in the national legislation.

There have been many advances in ecotoxicology since the definition of legal standards for metals and metalloids in freshwater in Brazil (18 years ago). For instance, the development of more sensitive analytical methodologies, allowing for the detection and quantification of lower concentrations of pollutants in environmental matrices; the development of *in vivo* and *in silico* approaches for the detection of toxic effects to aquatic organisms and quantification of toxic concentrations. Based on recent events of environmental pollution, such as mining dam collapses and data raised by ecotoxicologists, there is critical need to update legal standards to values that might be actually protective to the aquatic organisms.

Given these reasons, this study aimed to perform a spatiotemporal evaluation of ecotoxicological risks in the surface water of the Paraopeba River region affected by the B1 tailings dam collapse by using novel Predicted No Effect Concentration (PNEC) values defined by the Species Sensitivity Distribution (SSD) approach. This was done in order to (i) assess the efficiency of current environmental standards for the protection of biodiversity; (ii) assess short and long-term risks to aquatic organisms resulting from the B1 tailings dam failure, even for periods when ecotoxicological analyses were not conducted; and (iii) to help identifying priority areas or periods of concern in the watershed which require greater monitoring and recovery efforts. Furthermore, this study may stimulate the application of ecotoxicological risk assessment for the identification of critical areas in regions affected by tailings dam collapses or by other anthropic activities related to metal contamination by setting robust PNEC values.

2 GOALS

2.1 General Goal

To define novel PNEC values for metals and metalloids by constructing SSD curves for different trophic levels and to perform a spatial and temporal evaluation of ecotoxicological risks associated to these contaminants in the surface water of the Paraopeba River region affected by the B1 dam collapse at Mina Córrego do Feijão, in Brumadinho, Minas Gerais.

2.2 Specific Goals

- To define novel PNEC values for metals and metalloids based on SSD curves built for each trophic level;
- To evaluate current national and international legal standards related to metals and metalloids in freshwater according to their level of protection of biodiversity on the view novel PNEC values;
- To calculate ecotoxicological risks for each metal and metalloid in the Paraopeba River region affected by the dam collapse considering the most sensitive trophic level;
- To make a spatiotemporal evaluation of ecotoxicological risks to aquatic biota in the Paraopeba River region affected by the dam collapse in order to identify critical areas and periods for monitoring.

3 LITERATURE REVIEW

3.1 Mining and tailings dams

The beneficiation process in mining activities is performed to extract metals from the gangue (commercially worthless material) and generates different types of waste, such as sterile, soil, rocks and tailings. Tailings are defined as mixtures of crushed rocks, processing fluids from mills, washeries and concentrators (IBRAM, 2016; KOSSOFF *et al.*, 2014). The management of this waste is a great challenge to the mining industry worldwide due to its variable composition and high production volume. According to Islam and Murakami (2021), around 48,183 million cubic meters (Mm^3) of tailings are stored in 1,850 dams around the world, and this volume may reach 60,079 Mm^3 by 2025.

Different alternatives for final disposal or recycling of tailings are currently under development, such as the disposal in mining pits and dry stacks, or use as cementitious binders, hot asphalt filler or bricks (BEULAH *et al*., 2020; BASTIDAS-MARTÍNEZ et *al*., 2022; MARUTHUPANDIAN; CHALIASOU; KANELLOPOULOS, 2021; PINHO; FILHO, 2020; SCHOENBERGER, 2016). However, the disposal of tailings in dams is still the most common practice as it is, operationally, a low-cost solution since dams have high storage capacity (KALSNES *et al*., 2017; IPT, 2016).

There are at least three different constructions methods for dams' embankments raising: upstream, downstream, and center-line (vertically). In the first method, the embankment is raised at the beach of the previous level, within the existing damming by using tailings available at the area. In the downstream method, the embankment fill is made downstream the slope, outside the damming, whilst centerline is between the upstream and downstream method, where the new material is placed at the top of the embankment. In all methods, intermediate embankments are raised from the first initial dike construction as the storage demands advances, until the tailings dam full capacity is reached (KALSNES *et al.*, 2017; KOSSOFF *et al.*, 2014).

The upstream method is the most commonly applied for the construction of dams, corresponding to 50% of the tailings dams around the world. It is the cheapest, since tailings are used for embankment raising, thus reducing the consumption of raw material. Nevertheless, this is the less stable method, since it depends on the properties of the tailings discharged upstream (DO *et al.*, 2021). As upstream tailings correspond to the highest number of tailings dams worldwide and due to safety issues associated to this method, they also represent the most collapsed type around the world (57%) (ISLAM; MURAKAMI, 2021).

There are nearly 3,500 tailings dams spread worldwide (DO *et al.*, 2021; KOSSOFF *et al.*, 2014; LYU *et al.*, 2019). This number is an estimate due to lack of regulatory measures to ensure up-to-date records on tailings dam (ISLAM; MURAKAMI, 2021). According to the Brazilian Classification of Mining Dams defined by the Agência Nacional de Mineração (ANM) (The Brazilian Mining Agency), there are currently 923 tailings dams in Brazil, out of those, the biggest amount is located in Minas Gerais state (40%). Amongst these 923 Brazilian tailings dams, 58 are classified in the high-risk category due to technical properties, project conservation state or compliance with the dam's safety plan, out of which, 34 are in the state of Minas Gerais (ANA, 2021; ANM, 2023).

The higher number of tailings dams in Minas Gerais state, in comparison with other states, is a result of the historical role of Minas Gerais in the Brazilian mining scenario. The first gold ore deposits started to be discovered and exploited in the end of the 18th century, still during the Brazilian colonial period. This took place mainly the Quadrilátero Ferrífero (Iron Quadrangle) region. The exploitation of iron ore mining in this area gained importance by the end of the 19th century, with the gold mining decadence. The Quadrilátero Ferrífero is a mineral province and an economically active area of iron ore extraction, located at the central region of Minas Gerais state, mainly at Alto Rio das Velhas watershed, but also comprising Paraopeba River and Doce River basins (COTA; MAGALHÃES JÚNIOR, 2021). The iron ore present at Quadriláterio Ferrífero is associated with banded iron formations (itabirites) located on the São Francisco Craton southern border, an Archean granite greenstone terrain overgrown by sedimentary rocks from Proterozoic period and volcano-sedimentary rocks (PORSANI; DE JESUS; STANGARI, 2019).

When it comes to iron ore mining, each tone of processed product results in 0.4 tons of tailings. According to projections for the period between 2010 and 2030, iron ore beneficiation will contribute to 41% of tailings generated by mining companies in Brazil (IPT, 2016). Considering the number of iron ore mines in the Quadrilátero Ferrífero region, it is critical to impose strict tailings management practices and environmental monitoring programs.

Physicochemical properties of tailings originated from iron extraction vary according to the characteristics of the ore body, the beneficiation process, extraction efficiency and the degree tailings degradation in the dam. In general, iron and silica are the most abundant elements, as well as Mn, S, Mg, Al, K, Na and Ca (FREITAS *et al.*, 2019; KOSSOFF *et al.*, 2014). For instance, Vergilio *et al*. (2020) reported that tailings released from the B1 dam failure in Brumadinho were particulate materials (69.7% silt-clay and 0.3% sand), which contained Fe (264.9 mg g^{-1}), Al (10.8 mg g^{-1}), Mn (4.78 mg g^{-1}) and Ti (0.43 mg g^{-1}), among other elements. Trace toxic metals were also reported to occur in these tailings, such as U (1,457.4 μg g^{-1}), Sn (547.4 μg g^{-1}), Cd (30.94 μg g^{-1}), Pb (14.64 μg g^{-1}), As (4.69 μg g^{-1}), and Hg (101.3 ng g^{-1}). In contrast, Queiroz *et al*. (2018) listed Fe (452.00 ± 2.85 mg g^{-1}); Mn (0.43 ± 0.11 mg g^{-1}); Cr (63.9 ± 15.1 μg g^{-1}); Zn (62.4 ± 2.84 μg g^{-1}); Ni (24.7 ± 10.4 μg g^{-1}); Cu (21.3 ± 4.6 μg g^{-1}); Pb (20.2 ±4.6 μg g^{-1}) and Co (10.7 ± 4.8 μg g^{-1}) as the main components of Fundão iron ore tailings dam, which collapsed in Mariana in 2015.

The release of tailings in a watershed after a dam failure has been associated with environmental contamination of water and soil, leading to ecotoxicological effects on living organisms in impacted areas (PARENTE *et al.*, 2021; THOMPSON *et al.*, 2020; VERGILIO *et al.*, 2020).

3.2 Tailings dams collapses

Historically, tailings dams are more susceptible to failure than water dams due to different factors, such as: (i) the use of residual materials from mining operations (tailings, soil and coarse waste) to raise dam embankments, thus limiting material quality control and dam stability; (ii) constant dam raising elevation to increase storage capacity and volume; (iii) absence of regulatory policies related to project criteria; (iv) questionable quality control and monitoring measures to grant dam stability due to costs associated with periodic monitoring; (v) elevated costs of maintenance and remediation actions after mining closure, thus resulting in abandoned dams; and (vi) high risks assumed by managers to increase production levels while cutting costs (ARMSTRONG; PETTER; PETTER, 2019; AZAM; LI, 2010; ISLAM; MURAKAMI, 2021; RICO; BENITO; DÍEZ-HERRERO, 2008).

A study conducted by Islam and Murakami (2021) evaluated 366 incidents of tailings dam collapses which occurred around the world between 1915 and 2020. In this study, a rate of 3.45 ruptures per year was reported and a rising trend was detected. The United States was the country with the highest number of collapses (~115), probably due to its historical mining vocation and availability of data on public media. The authors also observed a shift in tailings dam collapses from developed nations to developing ones, as the number of cases in countries such as the US, the UK and Canada decreased after 2000, while cases in Brazil, China and Mexico increased. Besides, dams showing heights up to 50 m corresponded to 88% of the total number of collapses, and 54 out of the 366 incidents caused 2,976 human deaths.

The analysis of historical data from the WISE Uranium Project (2023) listed 153 major dam failures in the world between 1960 and 2022. The most recent was the rupture of a diamond tailings dam on November 7th, 2022 in Tanzania which released about 13 Mm^3 of tailings and water. In Brazil, 11 major tailings dam ruptures were reported, and 7 of them were in Minas Gerais. The last one occurred was the overflow of a sediment retention dam in the city Nova Lima, in January 2022.

The collapse of tailings dams may cause several environmental, economic and social impacts. The release of millions of tons of mud into water bodies and surrounding areas causes loss of biodiversity and native vegetation, as well as water quality impairement, such as increased suspended particulate matter (SPM) and metals concentration; reduced dissolved oxygen concentrations (KOSSOFF *et al*., 2014; SOARES *et al*., 2020; VERGILIO *et al*., 2021); and toxic effects to organisms from different trophic levels in water and sediments (PARENTE *et al.*, 2021; THOMPSON *et al.*, 2020; VERGILIO *et al.*, 2020). Besides environmental impacts, social impacts may also be highlighted, for example: loss of human lives and residences, restrictions for water usage (consumption, fishing and irrigation), and decrease in tourism activities (ISLAM; MURAKAMI, 2021; KOSSOFF *et al.*, 2014; VERGILIO *et al.*, 2020).

3.3 Vale S.A. tailings dam collapse in Brumadinho

Although previously classified as a low-risk dam by the Brazilian Mining Agency (VERGILIO *et al.*, 2020), Córrego do Feijão Mine Tailings Dam 1 (B1), owned by Vale S.A, collapsed on January 25th, 2019. The mining complex and dam are located in the municipality of Brumadinho, at the Metropolitan Area of Belo Horizonte (MABH), MG. This event entailed the following collapses of B-IV and B-IVA sediment retention dams. The B1 dam, built in 1974 and deactivated in 2016, had 86 m of height, 600 m of extension, a total area of 27 hectares and stored nearly 12 Mm^3 of iron ore tailings (DOMINGOS; CASTILHOS, 2019; FEAM; IEF; IGAM, 2021; IGAM, 2021; ROTTA *et al.*, 2020).

As a consequence of the collapse, the dam released about 10 Mm^3 of tailings into the Ferro-Carvão stream. Most of these tailings (7.8 Mm^3) were retained within 7 km downstream the Ferro-Carvão stream and the remaining volume reached the Paraopeba River (WISE URANIUM PROJECT, 2023). There is still no consensus on how far these tailings were transported through the river and monitoring studies are currently underway to estimate this distance. Despite that, according to the Two Years Book published by FEAM, IEF and IGAM (2021), tailings were transported for nearly 250 km until Retiro Baixo Hydroelectric Reservoir.

The tailings wave reached Vale's administrative area and the surrounding communities, and resulted in 270 human deaths, of which most were employees at the mining company and 3 are still disappeared (VALE, 2023; VERGILIO *et al.*, 2020). The release of tailings also compromised public water supply and destroyed 133.27 ha of Atlantic Forest vegetation and 70.65 ha of riparian areas (CIONEK *et al.*, 2019; PARENTE *et al.*, 2021). Regarding water quality in the Paraopeba River, recent studies have revealed the violation of legal standards set by the Brazilian legislation (BRASIL, 2005) for metals such as Fe (0.3 mg L^{-1}), Mn (0.1 mg L^{-1}), Al (0.1 mg L^{-1}), Zn (0.18 mg L^{-1}), Pb (0.01 mg L^{-1}), Cu (0.01 mg L^{-1}) and Cd (0.001 mg L^{-1}). Besides, increased turbidity and conductivity, reaching maximum values of 3,000 NTU and 273 µs cm^{-1}, respectively, were observed five days after the dam collapse. Concerning the sediments of Paraopeba River, Ni, Cu, Cr and Cd trespassed the Threshold Effect Level (TEL) established as a standard by the United States National Oceanic and Atmospheric Administration (NOOA) (THOMPSON *et al.*, 2020).

Although most studies published so far on the impacts of this disaster focus on physicochemical parameters, acute and chronic toxicity were also observed for organisms from different trophic levels, such as the algae *Raphidocelis subcapitata*, the microcrustacean *Daphnia similis* and the fish *Danio rerio* (THOMPSON *et al.*, 2020; VERGILIO *et al.*, 2020). The inclusion of ecotoxicological assays in monitoring plans following these events is critical to evaluate the ecological status of the water body summited to such sudden changes in physicochemical properties.

3.4 Ecotoxicology and water quality monitoring

Ecotoxicology is of great importance in environmental monitoring programs, especially following disasters. Ecotoxicological bioassays enable the detection and quantification of effects of single substances or environmental samples to test organisms through acute and/or chronic exposure assays. The difference between acute and chronic responses are related to the exposure period and endpoints or responses observed in each type of assay (MAGALHÃES; FERRÃO FILHO, 2008 apud SCHVARSTMAN, 1991).

Acute toxicity assays usually evaluate lethality or reactions of organisms right before death, such as immobility. In these assays, responses are severe and rapid, and occur as a consequence of exposure to high concentrations of a toxic compound/environmental sample in a short interval of time, from 0 to 96 hours depending on the life cycle of the test organism (MAGALHÃES; FERRÃO FILHO, 2008 apud RAND; PETROCELLI, 1985). The aim of the acute ecotoxicological test is to determine the dilution (or concentration) of a toxicant which causes death to 50% of all test organisms. This effect is expressed as LC_{50} (median lethal concentration) and is associated to the test duration, test organism and life cycle stage of the species. For some test organisms, the endpoint of the acute toxicity assay is not death, yet another effect (for example, luminescence decay is assessed as a response for acute toxicity to bacteria *Allivibrio fischeri*). In this case, results are expressed as the effect concentration which causes the observed effect to 50% of the population, thus named EC_{50} (median effect concentration) (COSTA *et al*., 2008; US EPA, 1994).

Although LC_{50} and EC_{50} are important thresholds to be used as a reference value for toxic effects, they refer to a given (and brief) exposure time established for each test.

Thus, if a substance moves slowly in tissues, it may probably show reduced toxicity for the period corresponding to the test, simply because its concentration in the target tissue has not reached sufficient levels to cause a toxic effect during the considered test time (COSTA *et al.*, 2008). Therefore, LC_{50} or EC_{50} values obtained during a longer exposure period are usually lower than those obtained in a short exposure time.

In contrast, chronic toxicity assays are generally longer than acute ones, lasting for at least 1/10 of the test organism life cycle. During a chronic assay, test organisms are exposed to reduced/sublethal concentrations of toxicants/environmental samples through a dilution series. Sublethal endpoints evaluated during chronic toxicity tests include changes in behavior, lack of mobility, growth reduction, defects in eggs development, reproduction issues, and nerve function commitment (US EPA, 1994; MAGALHÃES; FERRÃO FILHO, 2008). The response of a chronic assay is usually expressed in terms of NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) and EC_{10} (effect concentration to 10% of evaluated organisms). The NOEC is the highest concentration (or lowest dilution) of the substance/mixture that does not cause a statistically significant effect to test organisms at the standard exposure time and test conditions. The LOEC is the lowest concentration (or highest dilution) that causes a statistically significant effect to test organisms at standard exposure time and test conditions (COSTA *et al*., 2008; US EPA, 1994; NATH; DE; ROY, 2022). Similarly to the EC_{50}, the EC_{10} is the concentration which causes the observed effect to 10% of the population.

Among all these results derived from ecotoxicological assays, NOEC values are usually explored for the proposition of environmental standards aiming at the protection of aquatic fauna from toxic effects promoted by environmental pollutants, as they are derived from chronic toxicity assays. However, it is important to highlight that NOEC is calculated for a specific endpoint which does not eliminate possible occurrence of other effects below that concentration. Besides, these NOEC values are set based on the exposure of test organisms to single substances, thus failing to reproduce the complexity of the mixture of natural and exogenous substances present simultaneously in environmental samples.

3.4.1 Water quality regulations applied to ecotoxicity

In Brazil, the ecotoxicological control of surface water and effluents became first explicit in legal instruments in 2005, with the CONAMA Resolution n° 357/05 (BRASIL, 2005). This resolution disposes about the classification of water bodies and environmental guidelines for their framework and establishes conditions and standards for effluent discharge. According to this resolution, freshwater bodies are classified as special or classes 1, 2, 3 or 4, and water quality decreases as the number of the class increases. Saline and brackish water bodies are classified as special, or classes 1, 2 or 3.

CONAMA Resolution n° 357/05 (BRASIL, 2005) establishes that possible interactions between unregulated substances or contaminants which are likely to cause damage to living organisms should be submitted to ecotoxicological or toxicological tests (article 8th, paragraph 4). In addition, it determines that chronic toxic effects should not be observed in class 1 nor 2 freshwater bodies, or even in class 1 saline or brackish water bodies. For class 3 freshwater bodies, or class 2 saline or brackish water bodies, no acute toxic effect should be observed. The Resolution also establishes that an effluent should not cause or have potential to cause toxic effects to aquatic organisms (article 34th).

In 2011, the Resolution n° 357/05 was complemented and altered by the CONAMA Resolution n° 430/11 (BRASIL, 2011), which establishes that an effluent should not cause or have potential to cause toxic effects to aquatic organisms in the receptor water body as according to tests performed using organisms from at least two trophic levels.

For Minas Gerais state, the joint Normative Deliberation (ND) n° 08/22 from Conselho Estadual de Política Ambiental (COPAM) and Conselho Estadual de Recursos Hídricos (CERH) (MINAS GERAIS, 2022) complements directions imposed by CONAMA Resolutions n° 357/05 and n° 430/11. This normative proposes that ecotoxicological, toxicological, bioaccumulation and endocrine effects should be estimated by bioassays to investigate interactions between substances or the occurrence of contaminants which are capable to cause damage to living organisms.

It also states that waters classified as 1, 2 and 3 freshwaters must not present acute nor chronic toxic effect.

Beyond legislation, different standards define methodologies for ecotoxicological tests with a vast number of aquatic organisms. It is critical to use standardized assays, since results can be compared, and procedures may be reproduced. Table 1 shows some of the main ecotoxicological assays for freshwater recognized by the Associação Brasileira de Normas Técnicas (ABNT).

Table 1 – ABNT ecotoxicological tests standards for freshwater

Standard	Test organism	Matrix	Effect
ABNT NBR 12,716:1993 - Water - Acute toxicity test with fish - Part III - Flow through method - Method of test	*Cheirodon notomelas, Hemigrammus marginatus, Poecilia reticulata* or others from *Characidae* family	Freshwater	Acute
ABNT NBR 12,713:2016 - Aquatic ecotoxicology - Acute toxicity - Test with *Daphnia* spp (Cladocera, Crustacea)	*Daphnia similis*, *Daphnia magna*	Freshwater	Acute
ABNT NBR 15,088:2016 – Aquatic ecotoxicology - Acute toxicity - Test with fish (Cyprinidae)	*Danio rerio*, *Pimephales promelas*	Freshwater	Acute
ABNT NBR 15,499:2016 – Aquatic ecotoxicology - Short-term chronic toxicity - Test with fish	*Danio rerio*, *Pimephales promelas*	Freshwater	Chronic
ABNT NBR 13,373:2017 - Aquatic ecotoxicology - Chronic toxicity - Test method with *Ceriodaphnia* spp. (Crustacea, Cladocera)	*Ceriodaphnia dubia* *Ceriodaphnia silvestrii*	Freshwater	Chronic
ABNT NBR 12,648:2018 - Aquatic ecotoxicology - Chronic toxicity - Test with algae (Chlorophyceae)	*Chlorella vulgaris, Desmodesmus subspicatus, Raphidocelis subcaptata*	Freshwater	Chronic
ABNT NBR 15,411-1:2021 – Aquatic ecotoxicology - Inhibitory effect on *Vibrio fischeri* bioluminenscence - Part 1: Method using freshly prepared bacterias	*Vibrio fischeri*	Freshwater	Acute
ABNT NBR 15,411-2:2021 – Ecotoxicology aquatic - Inhibitory effect on *Vibrio fischeri* bioluminenscence - Part 2: Method using liquid-dried bacterias	*Vibrio fischeri*	Freshwater	Acute
ABNT NBR 15,411-3:2021 – Ecotoxicology aquatic - Inhibitory effect on *Vibrio fischeri* bioluminenscence - Part 3: Method using freeze-dried bacterias	*Vibrio fischeri*	Fresh, marine or estuarine water	Acute
ABNT NBR 15,469:2021 – Aquatic Ecotoxicology – Collection, preservation and preparation of samples	-	-	-

Source: adapted from Bertoletti (2013); Costa and Olivi (2008) and Jacob (2017).

Besides general rules associated to toxic effects promoted by environmental samples (surface water and wastewater), the Brazilian legislation on water quality (CONAMA Resolution n° 357/05) (BRASIL, 2005) specifies standards for a range of substances defined to contemplate the most restrictive value amongst all water uses considered for each class – drinking water, aquatic life protection, livestock production, irrigation and recreation (UMBUZEIRO; KUMMROW; REI, 2010). In some cases, these values were imported from other countries without any other rationale applied for their definition. This may fail to reflect the Brazilian reality, such as water temperature, water consumption rates and time spent in water recreation activities (UMBUZEIRO, 2015). Regarding Minas Gerais, values stablished by the joint Normative Deliberation COPAM/CERH n° 08/22 (MINAS GERAIS, 2022) are the same as those defined in the CONAMA Resolution n° 357/05 for metals and metalloids even despite its recent publication, in November 2022.

Despite scientific advances achieved over the 18 years since the definition of the limits of both regulations regarding metals and metalloids, they were not revised. Regarding aquatic life protection, ecotoxicological test standards have been revised in the past years and other countries have updated their water quality standards for aquatic life protection, such as the National Ambient Water Quality Criteria in the USA and the Canadian Water Quality Guidelines for the Protection of Aquatic Life (Canada) (CCME, 2022; US EPA, 2023); and many studies were conducted to define toxic concentrations of compounds to aquatic organisms (ARAMBAWATTA-LEKAMGE; PATHIRATNE; RATHNAYAKE, 2021; LIMA *et al.*, 2019). In addition, numerous chemical compounds have been developed. Thus, limits defined 18 years ago may not be effective for biodiversity protection.

3.4.2 Ecotoxicological assays applied to assess impacts of environmental contamination by mining tailings

Ecotoxicological evaluations are generally conducted for one or two species which correspond to a maximum of two trophic levels. The assessment of effects to different trophic levels in ecotoxicity assessments ensures a more robust analysis of ecosystem quality, since organisms belonging to different niche respond differently to chemicals, and environmental integrity relies on the interaction between organisms within the trophic chain and with the environment (PANDEY *et al.*, 2019; VERGILIO *et al.*, 2020).

Different ecotoxicological assays performed with organisms from distinct trophic levels have been used for ecotoxicological evaluation related to environmental contamination by mining tailings. Table 2 summarizes experimental conditions used for some of these assays. It is noted that most studies were conducted with one or two sample campaigns and did not evaluate the evolution of the toxicity over time. In addition, most studies were conducted with invertebrates and only a few of them with producers, the base of the food chain, which being affected by an event of contamination can influence the whole ecological equilibrium of the impacted area.

All studies reported in Table 2 were conducted with raw surface water, except Segura *et al*. (2016) who filtered water samples. Performing assays with unfiltered surface water is essential to assess the real effect of events involving increased turbidity and suspended solid (SS) concentration, such as dam ruptures. Assays carried out with filtered samples may disguise the toxicity, since turbidity and SS can be the main cause of toxicity in this type of samples.

Table 2 – Test organisms used to assess ecotoxicological effects of mining tailings or water and sediment samples contaminated by tailings

Organism	Group/trophic level	Matrix	References	N° of Campaigns	Tailings	Standards
Ceriodaphnia dubia	Microcrustacean/ consumer	Water Sediment	(MENDES *et al.*, 2020) (BESSER *et al.*, 2009)	13 25	Iron ore Lead-zinc	ANBT NBR 13,373/2017 ASTM and USEPA
Daphnia similis	Microcrustacean/consumer	Water Water/Sediment	(VERGILIO et al., 2020) (MENDES *et al.*, 2020) (VERGILIO *et al.*, 2021)	1 13 2	Iron ore	ABNT NBR 12,713/2009
Geophagus brasiliensis	Fish/consumer	Water	(GOMES *et al.*, 2019)	2	Iron ore	-
Raphidocelis subcapitata	Green algae/producer	Water/Sediment	(VERGILIO *et al.*, 2020) (VERGILIO *et al.*, 2021)	1 2	Iron ore	ABNT NBR 12,648/2018
Vibrio fischeri	Bacteria/ decomposer	Water	(MENDES *et al.*, 2020)	13	Iron ore	ABNT NBR 15,411-3/2011
Danio rerio	Fish/consumer	Water/Sediment Iron ore tailings suspension	(VERGILIO *et al.*, 2020) (MENDES *et al.*, 2020) (THOMPSON *et al.*, 2020) (VERGILIO *et al.*, 2021) (ALMEIDA *et al.*, 2021)	1 13 2 2 1	Iron ore	ABNT NBR 15,088/2016 -
Hyalella azteca	Amphipod/consumer	Sediment	(RIBA *et al.*, 2006) (BESSER *et al.*, 2009)	1 25	Pirite-Zinc ore Lead-zinc	Environment Canada guidelines ASTM and USEPA
Chironomus riparius	Chironomid/consumer	Sediment	(RIBA *et al.*, 2006)	1	Pirite-Zinc ore	Environment Canada guidelines
Tubifex tubifex	Oligochaete worm/decomposer	Sediment	(RIBA *et al.*, 2006)	1	Pirite-Zinc ore	Environment Canada guidelines
Proisotoma minuta	Collembola/consumer	Soil	(BUCH *et al.*, 2021)	2	Iron ore	ABNT NBR 17,512-1/2011; ABNT NBR 11,267/2014
Scheloribates praeincisus	Microarthropod/consumer	Soil	(BUCH *et al.*, 2020)	2	Iron ore	OECD guidelines
Allium cepa	Plant/ producer	Water Water, Mud/Soil Water/Sediment	(QUADRA *et al.*, 2019) (SEGURA *et al.*, 2016) (SOUZA *et al.*, 2021)	1 1 1	Iron Ore Iron Ore Iron Ore	- Modified Grant's protocol Modified Grant's protocol
Hoplias intermedius	Fish/consumer	Water/Sediment	(WEBER *et al.*, 2020)	2	Iron Ore	-
Hypostomus affinis	Fish/consumer	Water/Sediment	(WEBER *et al.*, 2020)	2	Iron Ore	-

3.5 Ecotoxicological risk assessment

Ecotoxicological assays are important tools for environmental quality monitoring and impact assessment, since they allow for the identification and quantification of the effects of natural or synthetic compounds and environmental samples upon living organisms, populations and communities from different trophic levels and environmental compartments (terrestrial or aquatic). Besides, they enable the assessment of effects corresponding to interactions between different compounds and mixtures present in the environment (MAGALHÃES; FERRÃO FILHO, 2008; REISS *et al.*, 2021). However, they demand time and resources, which can make long-term monitoring more challenging. Therefore, it is not always possible to carry out these assays.

In case it is not possible to get samples quickly and frequently enough to detect constant changes occurring in a watershed regarding toxic effects, especially considering the contamination of lotic systems, indirect methodologies, such as ecotoxicological risk assessment, may allow for the identification of critical areas for ecotoxicological sampling and monitoring. In this method, sampling is performed for the quantification of chemical compounds and Measured Environmental Concentration (MEC) is divided by Predicted No Effect Concentration (PNEC) reported in the literature for each compound to calculate the Risk Quotient (RQ) (RQ = MEC/PNEC). PNEC is defined as the concentration of a toxic compound below which most of the exposed organisms and ecosystem functions are unlikely to suffer unacceptable damage (DI LORENZO *et al.*, 2018; YOUNG; CHEN; YANG, 2021). A RQ below one corresponds to low ecotoxicological risk as, in this case, the concentration of a substance in the environment (MEC) is lower than PNEC (EUROPEAN COMMISSION, 2003). In contrast, a RQ above one means that the concentration of a certain substance in the environment is above the level considered to be safe to aquatic biota, thus representing an ecological risk.

Since the PNEC does not reflect the effect of a chemical to all species present in an environment, it is generally derived from extrapolations of data obtained from ecotoxicological assays performed with a limited number of species at an individual level (DEL SIGNORE *et al.*, 2016). There are two main ways to derive PNEC through extrapolation of laboratory data: (i) the deterministic method through which an assessment factor (AF) is applied, or (ii) the probabilistic method using Species Sensitivity Distribution (SSD) (BELANGER *et al.*, 2017; SORGOG; KAMO, 2019).

In the deterministic method, the PNEC is derived by dividing the lowest value reported for acute (Lethal concentration - LC_{50} or Effect Concentration - EC_{50}) or chronic toxicity (No Observed Effect Concentration - NOEC or Effect Concentration - EC_{10}) by an Assessment Factor (AF). The AF is used to account for uncertainties of extrapolating single-species laboratory results to a multi-species ecosystem and may vary from 10 to 1,000 depending on the considered effect (chronic or acute) and the number and quality of available ecotoxicity data, as presented in Table 3 (REIS; SANTOS; LANGE, 2021 apud EUROPEAN COMMISSION, 2003).

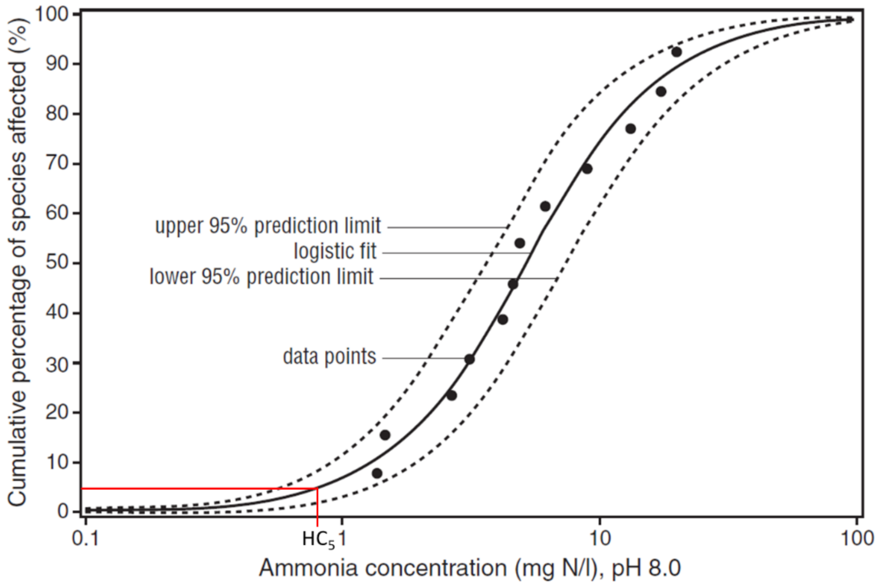
Table 3 – Derivation of acute and chronic PNEC values based on the deterministic method

Effect	PNEC assessment
Acute	$PNEC_a = \frac{LC_{50}\ or\ EC_{50}}{1{,}000}$
Chronic	$PNEC_c = \frac{NOEC\ or\ EC_{10}}{10}$

Source: adapted from European Commission (2003)

In contrast, the statistical SSD method allows for the estimation of a chemical concentration which represents hazard to 5% (HC_5) of species used in ecotoxicity assays. The HC_5 is calculated as the fifth percentile of a cumulative distribution curve (SSD curve) built with NOECs, EC_{10} or $L(E)C_{50}$ values for different species (Figure 1) (ALDENBERG and JAROWSKA, 2000). In this method, the PNEC threshold is determined by dividing the HC_5 value by an AF that varies from 1 to 5 depending on, for example, the database quality, and the diversity and representativity of taxonomic groups in a database (EUROPEAN COMMISSION, 2011; SORGOG; KAMO, 2019).

Figure 1 - Example of ammonia SSD curve. Solid line is the regression model fit to data, broken lines are 95% prediction limits and in red is the HC_5 value



Source: Posthuma, Sutter II and Trass (2002).

Each of these methods has advantages and limitations. Although the deterministic method can be applied for any sample size, it only considers one specie - the most sensitive according to ecotoxicity results available in the literature - for the calculation of PNEC. This method overlooks the fact that a more sensitive specie which has never been used as a test organism may occur in a certain environment. Meanwhile, the SSD method considers the ecotoxicological risk uncertainties (FOX *et al.*, 2021; SORGOG; KAMO, 2019) as PNEC value derived from this method is based on a minimum of 10 different species, according to Belanger and Carr (2019). As reported by Sorgog and Kamo (2019), the performance of both methods increases as the number of species rises, but the SSD method tends to be recommended when there is a higher variability among toxicity data (standard deviation > 0.9). However, there is no consensus of which method is the best, mainly due to the absence of comparison studies.

The SSD approach has become widely applied in the United States, Canada, Australia, New Zealand and in the European Union, as a method for derivation of water quality criteria, for the estimation of ecological risk and for the characterization of chemical contaminants effects on water quality. In Canada, for instance, SSD is the method recommended by the Canadian Water Quality Guidelines for the Protection of Aquatic Life (DEL SIGNORE *et al.*, 2016; FOX *et al.*, 2021; SORGOG; KAMO, 2019). In Brazil, the SSD methodology is being applied in scientific researches, mainly in order to compare laboratory toxicity observed to specific organisms to other aquatic specie (ALHO *et al.*, 2022; GEBARA *et al*., 2020). However, the SSD approach is not yet applied for the derivation of water quality standards in Brazil.

3.5.1 Bioavailability of metals in ecotoxicological risk assessment

In aquatic toxicology, the extent or rate to which a substance reaches the toxic site of action is defined as bioavailability (ADAMS *et al.*, 2020). This aspect is essential for ecotoxicological risk evaluations as it directly reflects the fraction of the substance in the environment that is available for biological uptake, which is usually low for metals under natural environmental conditions in aquatic environments (LATHOURI; KORRE, 2015). Thus, bioavailability has a direct effect on results obtained in ecotoxicological risk assessment involving metals.

Generally, the more soluble a metal, the more bioavailable and toxic it is. However, the bioavailability of metals in water is a complex aspect and does not rely only on the quantification of total and dissolved fractions of metals (MAGALHÃES *et al.*, 2015). More than that, bioavailability involves many aspects of water chemistry such as pH, redox potential, hardness, dissolved oxygen concentration, sulfides, alkalinity and natural organic matter content. These factors may affect the metal solubility, oxidation state, capacity of complexation with organic compounds and metal speciation. As a result, bioavailability may vary, influencing the capacity of metals to interact with aquatic organisms resulting in toxic effects (ADAMS *et al.*, 2020; VÄÄNÄNEN *et al.*, 2018).

One of the most important variables that affects metals speciation in the environment is the pH as it determines de degree of solubility, hydrolysis, precipitation, coagulation and proton competition for available ligands. Normally, metals solubility, and consequently bioavailability, is decreased in higher pH, since metals tend to precipitate as oxides and hydroxides, except for Al^{3+} and Ba^{2+}, the alkali metal hydroxides (MAGALHÃES *et al.*, 2015). However, there is no general rule for how pH affects metals bioavailability. For example, Grosell, Gerdes and Brix (2006) observed higher toxicity of Pb in the fish *Pimephales promelas* at higher pH, due to lower competition between the metal and H^+ at the gill surface. This makes the metal more available to be absorbed by the gill, besides lower fraction of bioavailable metal in the environment at higher pH values.

Besides pH, hardness also plays a key role on metals bioavailability. Higher concentration of Ca^{2+} and Mg^{2+} tends to decrease metals toxicity, since these cations compete with divalent metals ions for sensitive receptors in the organism (LATHOURI; KORRE, 2015; MAGALHÃES *et al.*, 2015). Alkalinity, besides many times directly linked with hardness and pH, influences metal speciation through metal complexation with carbonate and bicarbonate ions. Thus, higher alkalinity reduces free metals in aqueous solution, and consequently, decreases bioavailability and toxicity (LATHOURI; KORRE, 2015).

The presence of natural (humic and fulvic acids) or anthropogenic sources of organic carbon mainly via organic ions, may reduce the concentration of free metals ions and, consequently, their bioavailability. This is due to the complexation of metals and organic ions, but also due to adsorption or cation exchange. Humic substances present linking groups to which metals can bind in a relatively stable condition, depending on the pH, complexation time, photodegradation and concentration of these aquatic humic substances (MAGALHÃES *et al.*, 2015)

As it is difficult to measure bioavailable concentration of a metal in the aquatic environment, this might be estimated by using appropriate computational models for which input data are the dissolved concentration of a metal and some water physicochemical parameters (LATHOURI; KORRE, 2015). Biotic Ligand Model (BLM) is one of the available models used to predict metal toxicity and has been widely adopted in Europe and in the United States (US) for the definition of water quality standards and for regional risk assessments (BRIX *et al.*, 2021). In the BLM, the strength of ligand affinity is used to model the reactions that occur between the metal of interest and cations with different available ligands that naturally occur in water. One of these ligands is the biotic ligand. When metals bind with these biotic ligands (physiological active site of toxic action), ion regulatory process can be disrupted, causing toxic effects to the cell (ADAMS *et al.*, 2020; LATHOURI; KORRE, 2015). The concentrations of metal-ligand complexes and free metals are predicted based on the combination of the interest metal and water chemistry parameters. The median acute effect data can be estimated, for example, when the concentration of the metal in the biotic ligand (accumulation concentration, LA) reaches the concentration associated with 50% of mortality (ADAMS *et al.*, 2020; MEBANE, 2022).

The original BLM models require different water chemistry parameters (at least 8, depending on the metal of interest) as input. For instance, parameters such as temperature, pH, calcium, sodium, magnesium, potassium, alkalinity, sulfate, sulfide, chloride, Dissolved Organic Carbon (DOC) and fraction of DOC as fulvic and humic acids, are required for estimating Cu bioavailability (MEBANE, 2022). Thus, BLM has been adopted by a few regulatory institutions due to requirements associated to input data, models complexity, time required per sample and level of operator skills necessary to interpretate the outputs (WFD, 2014).

In order to overcome challenges associated to the application of BLM, different “user-friendly” bioavailability tools were developed. Although they were based on BLM, these tools require less input variables (only pH, Ca and DOC), resulting in faster simulations and reduced costs. The Bio-met bioavailability tool and the Metal Bioavailability Assessment Tool (M-BAT) are two examples of “user-friendly” tools developed based on BLM. Bio-met is a collaborative initiative led by the European Copper Institute, the International Zinc Association and the Nickel Institute NiPERA. This tool is currently available for Cu, Ni, Zn, Pb and Co bioavailability evaluation (BIO-MET, 2021). Similarly, M-BAT was developed by the United Kingdom Technical Advisory Group (UKTAG) within the scope of the Water Framework Directive to estimate the bioavailability of Cu, Zn, Mn and Ni (WFD, 2014).

Both Bio-met and M-BAT are algorithm-based tools developed in MS Excel which allow for the calculation of the Bioavailability Factor (BioF) - the bioavailability of a metal considering specific water chemistry characteristics. BioF is the ratio between the HC_5 obtained via SSD curves and the local HC_5 obtained under specific water conditions. The maximum ratio is 1, which corresponds to 100% bioavailability of a metal under the specific water conditions provided, that is named “sensitive condition” and demands specific attention regarding aquatic life protection (BIO-MET, 2021; LATHOURI; KORRE, 2015).

Although bioavailability is a fundamental factor in ecotoxicological assessments, there are only a few studies in the scienitific literature that consider this important aspect to build SSD curves. Lathouri and Korre (2015), for example, evaluated the influence of temperature into HC_5 values obtained for Cu, considering water quality of four rivers in the UK. The referred study observed lower HC_5 values in the cold season, not due to temperature effect in Cu speciation, yet due to its effect on pH, dissolved solids, alkalinity and organic carbon which affect bioavailability. Cu bioavailability varied markedly due to different water physicochemical characteristics and DOC was the most important factor for the bioavailability of this metal.

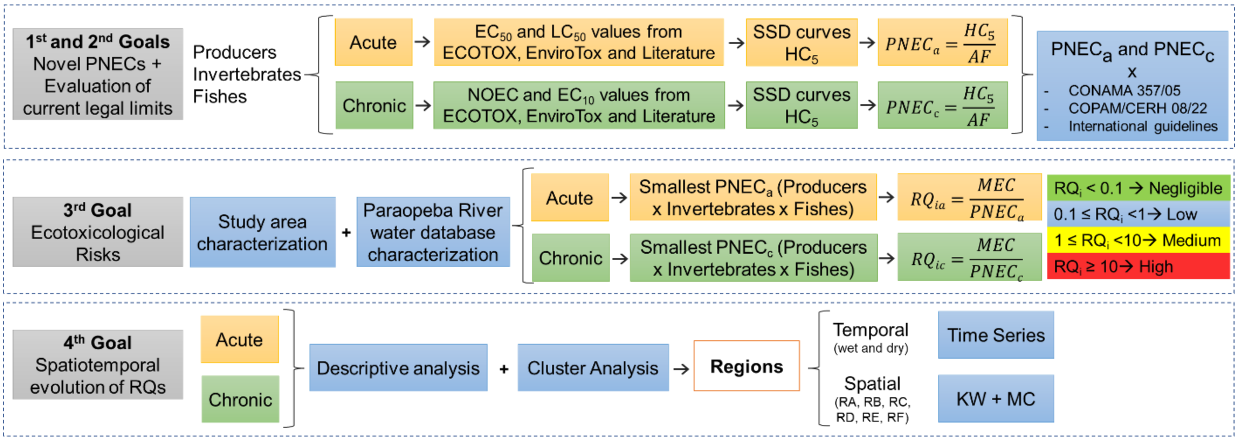
Mebane *et al*. (2020a) evaluated Cu, Cd and Zn toxicity to aquatic insect communities, based on SSD curves and considering bioavailability as according to water characteristics (pH 7.5, DOC 0.5 mg L^{-1}, 14 mg L^{-1}, and hardness 85 mg L^{-1} as $CaCO_3$) by using Bio-met. HC_5 values of 0.38, 2.42 and 10.9 g L^{-1}, respectively, were observed for Cd, Cu and Zn.

Results reported by Lathouri and Korre (2015) and Mebane *et al*. (2020a) confirm that water physicochemical characteristics influence the bioavailability of each metal in a different way and emphasize the necessity of more detailed studies which consider bioavailability and which investigate the effect of other parameters on bioavailability. Besides, there is critical need for studies conducted under natural conditions, where different factors, such as temperature, ionic strength of the medium, redox potential, flow rate and ecological relationships, might affect bioavailability, in contrast to studies conducted in laboratory-controlled environments in which test-organisms are exposed to synthetic solutions.

4 METHODOLOGY

Figure 2 shows a simplified schematic design of the different methodology stages used to achieve goals proposed in this study which are described in detail in the following items.

Figure 2 – Schematic design of the methodology applied in this study



KW = Kruskal-Wallis test; MC = multiple comparison test; RA = Region A; RB = Region B; RC = Region C; RD = Region D; RE = Region E; RF = Region F; RQ = Risk Quotient; AF: Assessment Factor; MEC: Measured Environmental Concentration; PNEC: Predicted No Effect Concentration.

Source: author (2023)

4.1 Study area

The present study was carried out in the Paraopeba River region affected by the collapse of Vale S.A. Company B1 tailings dam and the consequent collapses of B-IV and B-IVA sediment retention dams. The event occurred on January 25th, 2019, in the municipality of Brumadinho, at the central region of Minas Gerais state, about 65 km from Belo Horizonte (MG state capital) (PORSANI; DE JESUS; STANGARI, 2019; ROTTA *et al.*, 2020).

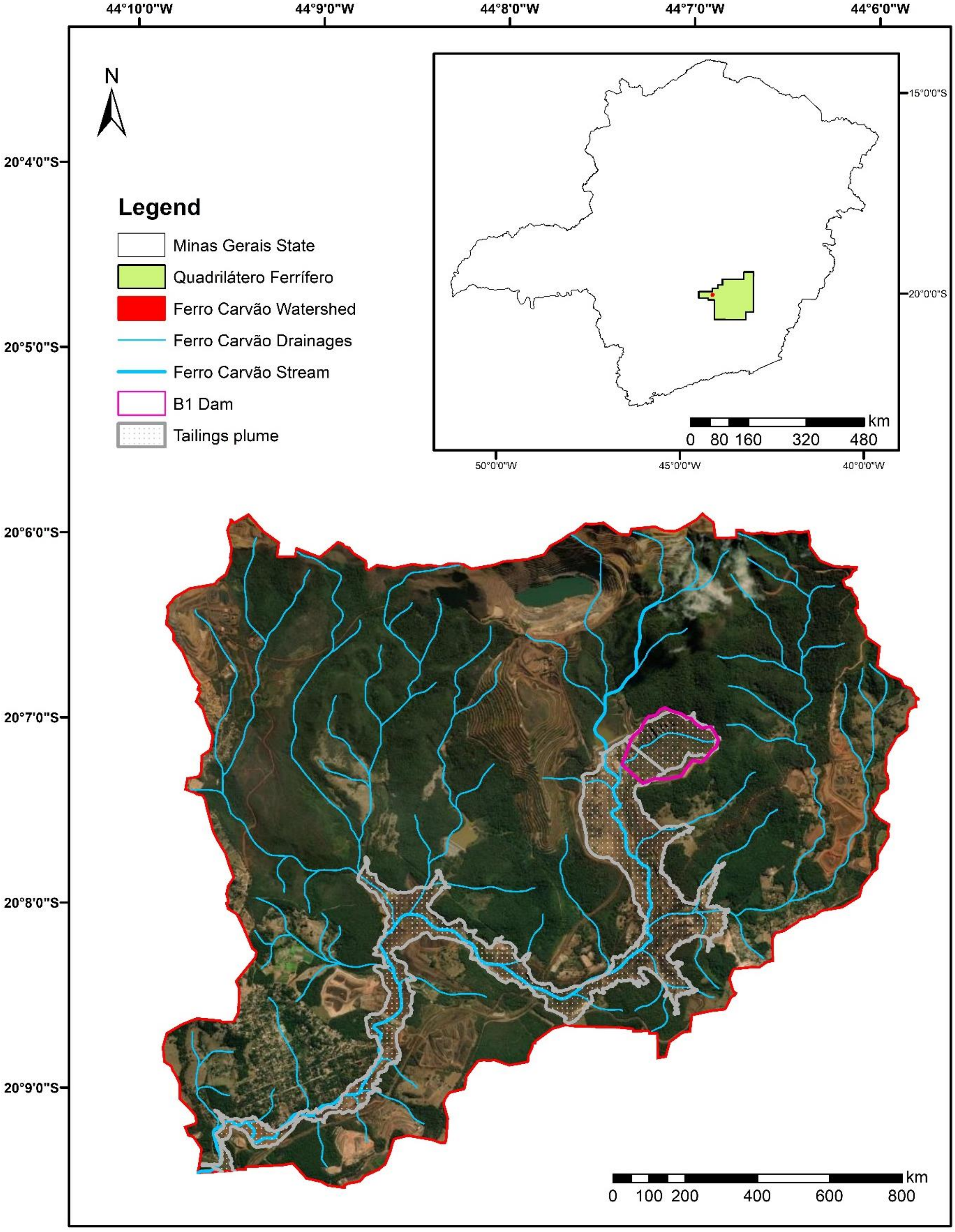
Despite its inactivation in 2016, B1 dam was part of Córrego do Feijão mine complex that also comprised a maintenance center, an administrative office, a cargo terminal, and a railway line for iron ore transportation. This mine complex produced 8.5 million tons of iron ore in 2018 and is located in the economically iron ore extraction region of the Quadrilátero Ferrífero (PORSANI; DE JESUS; STANGARI, 2019).

After the rupture, 10 Mm^3 of tailings reached Ferro Carvão stream, an affluent of the Paraopeba River (Figure 3). Ferro Carvão watershed comprises an area of 32.8 km^2 and presents a medium flow of 600 L s^{-1}. Nearly 8 Mm^3 of the released tailings were retained at the Ferro Carvão stream and about 2 Mm^3 reached the Paraopeba River, which is the major tributary of the São Francisco River and one of the tributaries of the Três Marias hydroelectric reservoir (CPRM, 2019; FEAM; IEF; IGAM, 2021). The Paraopeba River watershed occupies an area of 13,643 km^2 and covers 48 municipalities. It is an important source of water supply to more than 5.2 million inhabitants at the Metropolitan Area of Belo Horizonte (MABH) and Pará de Minas, while also providing water for irrigation and industries, mainly in the mining sector (CPRM, 2019; IGAM, 2019a).

Besides Três Marias reservoir, there are two other hydroelectric plants in the Paraopeba River, from upstream to downstream: Salto do Paraopeba and Retiro Baixo. Other water uses of the Paraopeba River watershed are irrigation and industrial water supply, mainly for mining (CPRM, 2019).

According to COPAM Normative Deliberation n° 14 from December 28th, 1995 (MINAS GERAIS, 1995), the Paraopeba River watershed is classified as Class 2 from the confluence with the Maranhão River until Três Marias dam, which encompasses the region under study. Considering this class, CONAMA Resolution n° 357/05 (BRASIL, 2005) and COPAM/CERH Normative Deliberation n° 08/22 (MINAS GERAIS, 2022), there should be no acute nor chronic toxic effects in surface water in this region, thus reinforcing the need for studies on ecological evaluation.

Figure 3 – Map showing the Ferro Carvão watershed, B1 dam and track of the tailings plume after the collapse in the context of Quadrilátero Ferrífero region in the state of Minas Gerais



Data source: Plataforma Brumadinho (2023); SISEMA (2023); UFOP (2023)
Geographical coordinates: SIRGAS 2000.
Map source: author (2023)

4.2 Paraopeba River water quality database

The present study was based on secondary data obtained within the scope of a monitoring program named “Programa de Monitoramento de Qualidade das Águas Superficiais e Sedimentos (PMQAS)”, conducted by the company Vale S.A. between January 26th, 2019 and February 14th, 2022 and provided by the National Water Agency (ANA – Agência Nacional de Águas e Saneamento Básico). The database comprised 25 sampling points located along the Paraopeba River from 10 km upstream the collapsed dam region until Três Marias reservoir (342 km downstream the collapse region), as presented in Figures 4 and 5. Table 4, presents distances from monitoring points to B1 tailings dam as well as kick-off and end dates for each point.

Surface water samples were collected and analyzed for metals, metalloids, anions, nutrients, Polychlorinated Biphenyls (PCBs), pesticides, biological indicators, Semi Volatile Organic Compounds (SVOC), Volatile Organic Compounds (VOC), Total Petroleum Hydrocarbons (TPH) and radioactive parameters. There was no regularity in monitoring frequency regarding stations and parameters, however, most of them was monitored daily. Concentration of dissolved metals and metalloids were the parameters of interest in this study.

Data associated to metals and metalloids in water – Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, U, Zn – were first evaluated according to total data, percentage of censored data (under the method limit of quantification - LOQ) and violation of legal limits. Parameters containing more than 80% of censored data were individually evaluated and censored data were replaced by LOQ values to enable calculations.

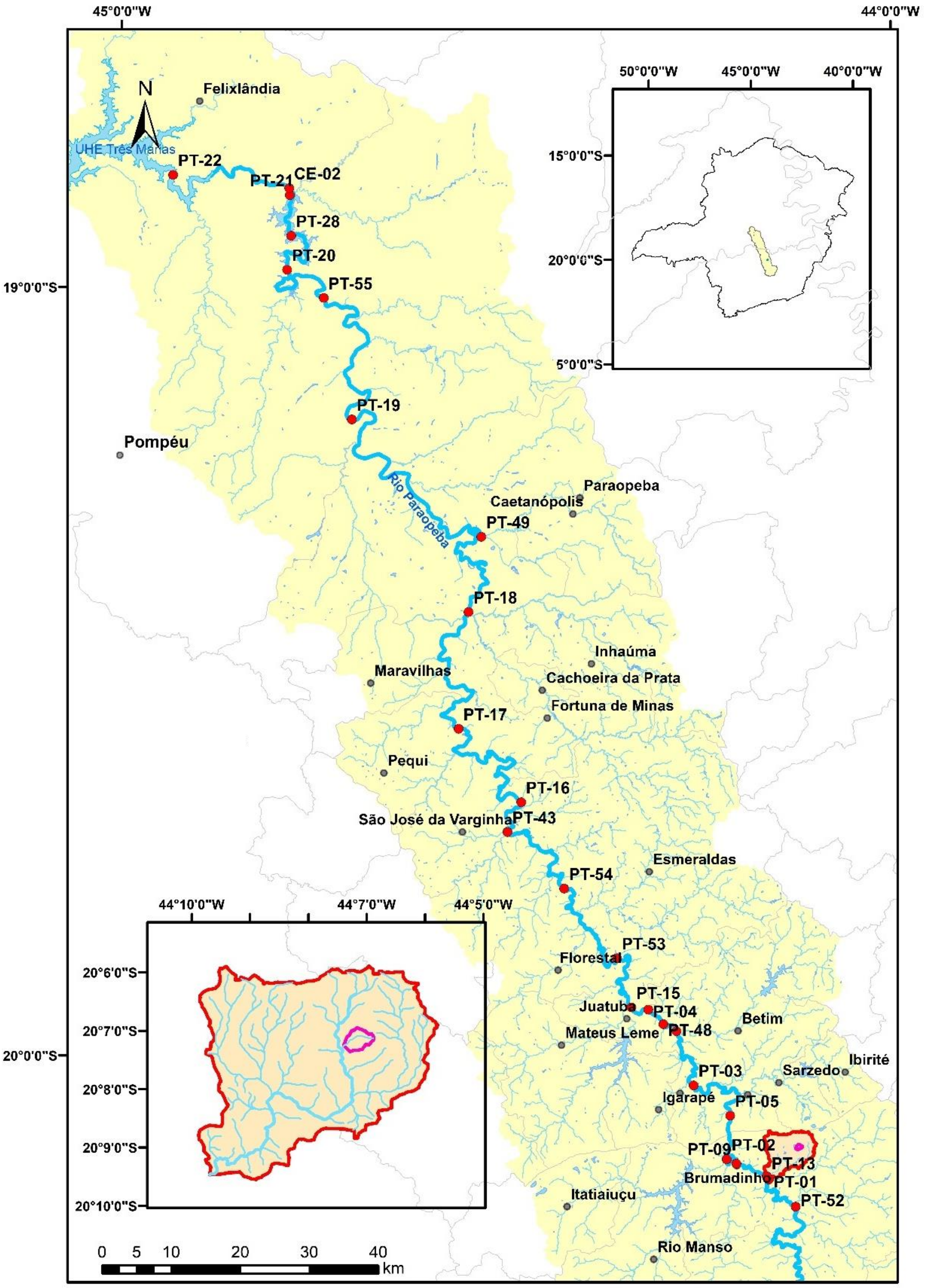
Table 4 – Location, monitoring period and distances of each monitoring point in PMQAS to the B1 tailings dam

Monitoring points	Approximate distance from/to B1 dam (km)	Coordinates		Location	Monitoring period	
		Latitude	Longitude		Kick-off date	End date
PT-52	-10.0	20°11'48.8"S	44°7'24.2"W	10 km upstream B1 dam – Brumadinho city	25/02/2019	14/02/2022
PT-01	-0.4	20°9'41.5"S	44°9'29.9"W	50 m upstream the dredged area by Vale S.A.	28/01/2019	14/02/2022
PT-13	0.2	20°9'26.9"S	44°9'39.8"W	200 m upstream the confluence with Ferro-Carvão Stream	15/05/2019	14/02/2022
PT-09	5.6	20°9'14.1"S	44°9'30.7"W	3.6 km downstream the dredged area by Vale S.A. – Brumadinho	28/01/2019	14/02/2022
PT-02	10.3	20°8'27.1"S	44°12'2.3"W	8.4 km downstream the dredged area by Vale S.A. – at COPASA	29/01/2019	14/02/2022
PT-05	17.5	20°8'7.2"S	44°12'47.0"W	15.3 km downstream the dredged area by Vale S.A.	31/01/2019	14/02/2022
PT-03	33.3	20°4'42.5"S	44°12'31.0"W	Upstream São Joaquim Creek (BR-381 bridge)	28/01/2019	14/02/2022
PT-14	45.6	20°2'20.6"S	44°15'22.1"W	Confluence with Betim River, close to Igaratermo termelectric	28/01/2019	25/01/2022
PT-48	48.3	19°58'6.6"S	44°16'42.9"W	Upstream Juatuba water catchment and downstream Ceriroca Creek	07/02/2019	09/02/2022
PT-04	51.6	19°57'34.0"S	44°17'44.0"W	Downstream Pimenta Creek, after Juatuba water catchment	28/01/2019	15/10/2021
PT-15	55.0	19°56'25.8"S	44°18'55.0"W	Upstream Serra Azul Stream	31/01/2019	14/02/2022
PT-53	68.4	19°56'15.1"S	44°20'13.4"W	Upstream Lavrinha Creek	25/02/2019	25/01/2022
PT-54	89.5	19°52'24.8"S	44°21'21.3"W	Downstream Lajinha Creek	27/02/2019	25/01/2022
PT-43	108.1	19°46'58.2"S	44°25'28.6"W	Para de Minas city water catchment	29/01/2019	25/01/2022
PT-16	115.0	19°42'33.7"S	44°29'54.1"W	Downstream Rancho Alegre Stream (MG 060 bridge)	26/01/2019	25/01/2022
PT-17	147.7	19°40'13.8"S	44°28'50.1"W	Downstream Laranjeiras Creek (MG-238 bridge)	26/01/2019	14/02/2022
PT-18	201.7	19°34'30.2"S	44°33'43.3"W	Downstream São João Stream	26/01/2019	14/02/2022
PT-49	221.9	19°25'22.7"S	44°32'56.8"W	Downstream Capão da Onça Creek	25/02/2019	10/02/2022
PT-19	265.3	19°19'30.3"S	44°31'57.0"W	Bridge of MG-420 road over Paraopeba River	26/01/2019	25/01/2022
PT-55	282.2	19°10'20.8"S	44°42'3.7"W	Usptream Velho River (Choro's Waterfall)	25/02/2019	14/02/2022
PT-20	297.7	19°0'51.1"S	44°44'15.2"W	Retiro Baixo Hydroelectric reservoir	26/01/2019	14/02/2022
PT-28	309.0	18°58'39.1"S	44°47'6.5"W	Retiro Baixo Hydroelectric Dam	31/01/2019	14/02/2022
PT-21	315.1	18°55'59.9"S	44°46'47.3"W	Retiro Baixo Dam spillway – Felixlândia	27/01/2019	14/02/2022
CE-02	316.2	18°52'49.0"S	44°46'54.2"W	4 km downstream Retiro Baixo Dam	23/02/2019	14/02/2022
PT-22	341.7	18°52'16.9"S	44°46'56.5"W	Três Marias reservoir backwater beginning	26/01/2019	14/02/2022

Negative values means that the point is located upstream the B1 dam.

Source: author (2023)

Figure 4 – Study area: Ferro Carvão stream basin and monitoring points in the Paraopeba River watershed



LEGEND

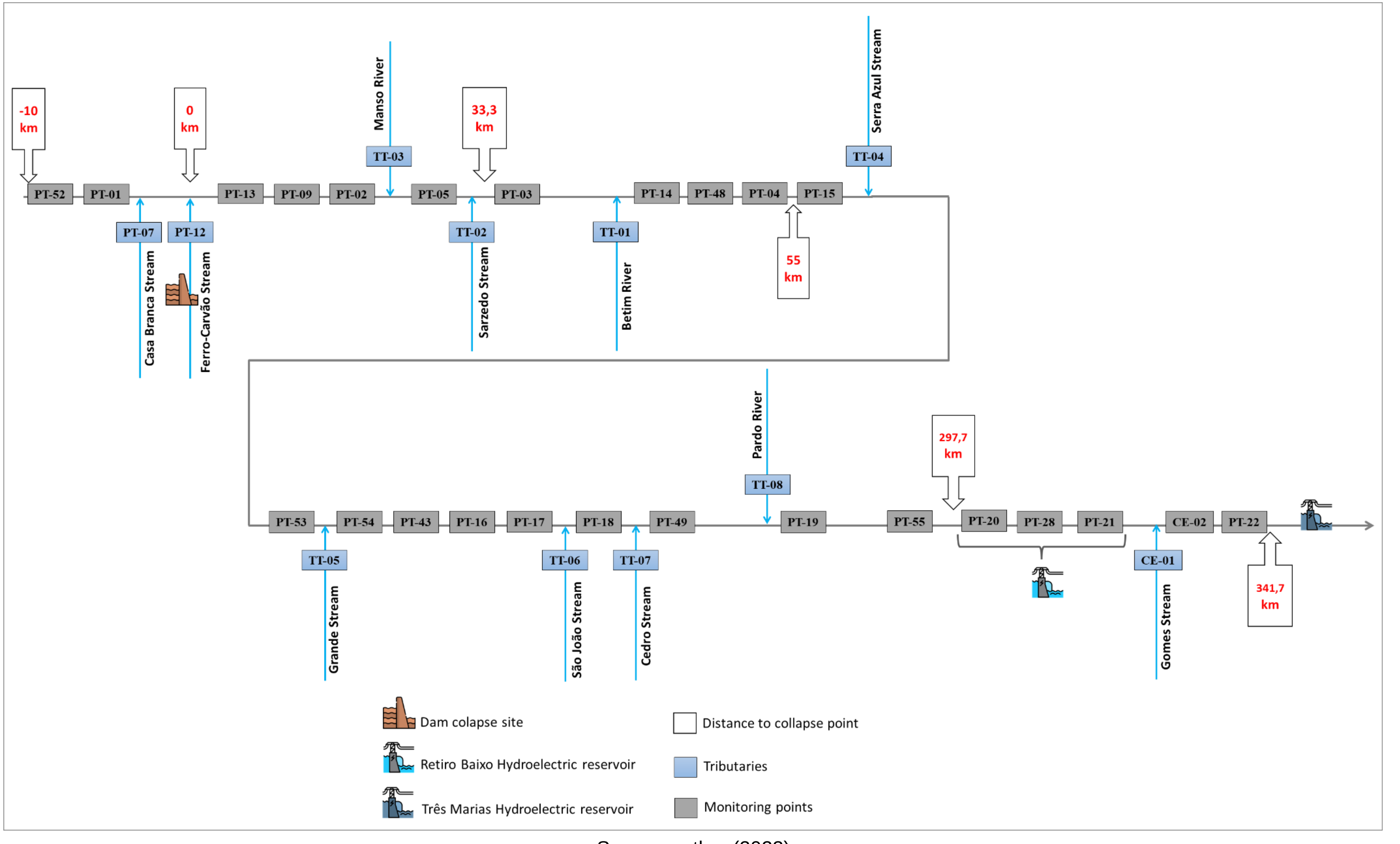
B1 Dam
PMQAS points
Ferro Carvão Watershed
Paraopeba Watershed
Main Drainages
Paraopeba River
Dams and lakes
State Boundary
Municipal Seats
Municipal Boundaries

Data source: Plataforma Brumadinho (2022); SISEMA (2023).

Geographical coordinates: SIRGAS 2000.

Map source: author (2023)

Figure 5 – Line diagram (upstream to downstream) of monitoring points in the Paraopeba River and tributaries



Source: author (2023)

4.3 Evaluation of legal standards using Species Sensitivity Distribution

In order to evaluate current legal standards set for metals and metalloids (Al, As, Cd, Pb, Co, Cu, Cr, Fe, Mn, Hg, Ni, Ag, U, Zn), PNEC values were estimated for acute and chronic effects and compared to legal limits set for freshwater worldwide: (i) Resolution CONAMA n° 357/05 (BRASIL, 2005); (ii) Normative Deliberation COPAM/CERH n° 08/22 (MINAS GERAIS, 2022); (iii) Environmental Quality Standards from the European Union (EU) (EUROPEAN PARLIAMENT, 2008); (iv) National Recommended Water Quality Criteria, from the United States of America (USA) (US EPA, 2023); (v) Canadian Water Quality Guidelines for Aquatic Protection Life (CCME, 2022); and (vi) Proposed (UK) (COMBER; GEORGES, 2008; JOHNSON *et al.*, 2007; LEPPER *et al.*, 2007; MAYCOCK *et al.*, 2007; MAYCOCK *et al.*, 2012; PETERS *et al.*, 2010).

To this end, acute and chronic Species Sensitivity Distribution (SSD) curves were constructed for each metal/metalloid for three trophic levels – producers, primary and secondary consumers – represented, respectively, by algae, invertebrates and fishes, based on ecotoxicity data (EC_{50} and LC_{50} for acute, and NOEC, EC_{10} and LOEC for chronic effects) (IWASAKI *et al.*, 2015). Ecotoxicity data were gathered from the EnviroTox database (CONNORS *et al.*, 2022), the US EPA ECOTOXicology knowledgebase (US EPA, 2022) and from general scientific databases for at least 7 different species of each trophic level. Data from EnviroTox and ECOTOX database published previously to 1980 was not considered due to lack of reliability in experimental and analytical techniques (PARK; KIM, 2020), except for chronic effects of As, Ag and Mn, for which older studies (post 1978) had to be considered in order to achieve at least 7 species per trophic level.

Papers published in recent years (last 10 years) were prioritized for the construction of the database and data published before this period was only used in case of lack of data. In general, it was not clear in papers if metal concentration was total or dissolved. Metal was considered as dissolved when a stock solution was prepared in order to conduct the toxicity test, however, it was not always clear which salt was used to prepare the stock solution. Besides generally standardized for toxicity assays, information regarding hardness and temperature conditions adopted during tests was also not always available in papers.

If more than one ecotoxicity value was obtained for the same specie, the geometric mean was calculated, in order to avoid overrepresentation (ARAMBAWATTA-LEKAMGE; PATHIRATNE; RATHNAYAKE, 2021).

The ETX software, version 2.3 (VAN VLAARDINGEN *et al.*, 2014), was used to construct SSD curves and to determine acute and chronic HC_5 values and their 95% confidence intervals for all the 14 metals/metalloids, based on the methodology developed by Aldenberg and Jarowska (2000). Since the ETX software assumes a log-normal distribution of ecotoxicity data, the log-normality was evaluated by the Kolmogorov Smirnov test (5% of significance) included in the software package (LIMA *et al.*, 2019).

For Hg and Mn chronic effect to algae, and U and As chronic effect to invertebrates, the chronic HC_5 value was calculated by dividing the acute HC_5 value by 10, since data for at least 7 different species were not available, as according to Hiki and Iwasaki (2020).

As bioavailability strongly influences the toxicity of metals and metalloids, and there are tools available to estimate their bioavailability and adjust HC_5 values according to water quality features. Bio-met and M-BAT software were used in this study to calculate the bioavailability of Cu, Ni, Zn, Pb and Co (BIO-MET, 2021) and Mn (WFD, 2014), respectively. Median values of pH (7.22), Dissolved Organic Carbon (DOC) (3 mg L^{-1}) and dissolved Calcium (5.91 mg L^{-1}) were calculated from the Paraopeba River water quality database and used as input values in the previously mentioned software to calculate the Bioavailabity Factor (BioF). Then, HC_5 bioavailable values (HC_{5bio}) were calculated as according to Equation 1, where HC_{5bio} is the HC_5 estimated considering the bioavailability; HC_5 is the hazard concentration for 5% of considered species obtained from SSD curves; and BioF is the Bioavailability Factor (BIO-MET, 2021).

$$HC_{5\mathrm{bio}} = \frac{HC_5}{BioF} \quad (1)$$

PNEC values for acute and chronic effects were calculated based in Equation 2, by dividing the value of HC_5 or HC_{5bio} (when available) by an assessment factor (AF) that considers the uncertainties of extrapolating laboratory results from a single specie to a multi-species in the environment (GREDELJ *et al.*, 2018; RAZAK *et al.*, 2021).

$$PNEC = \frac{HC_5 \ or \ HC_{5bio}}{AF} \tag{2}$$

The AF value applied in this study was 1 based on the criteria defined by the European Technical Guidance Document on Risk Assessment, and considering that a conservative approach was adopted to construct the SSD curves for three different trophic levels (EUROPEAN COMMISSION, 2003; LATHOURI; KORRE, 2015; RAZAK *et al.*, 2021).

4.4 Ecotoxicological risk assessment

Ecotoxicological risks for acute and chronic effects associated to each metal and metalloid in the Paraopeba River were assessed based on the Risk Quotient (RQ_i) as shown in Equation 3.

$$RQ_i = \frac{MEC_i}{PNEC_i} \tag{3}$$

where MEC is the measured concentration of the dissolved metal/metalloid in the PMQAS scope; PNEC is the Predicted No Effect Concentration previously determined based on the SSD curves and on bioavailability evaluation; and i refers to each metal or metalloid evaluated.

Chronic and acute RQ_i values for each metal and metalloid were calculated for each monitoring point and considering all observations obtained during the monitoring period based on the most sensitive trophic level per metal/metalloid, defined by the smallest PNEC values.

Once obtained, RQ values were classified into four categories, according to YOUNG, CHEN and YANG (2021): (i) RQ < 0.1 – negligible risk; (ii) 0.1 ≤ RQ < 1.0 – low risk; (iii) 1 ≤ RQ < 10 moderate risk and (iv) RQ ≥ 10 high potential risk. A descriptive analysis of RQ was conducted for each metal/metalloid, based on minimum, 1st and 3rd quartiles, median, mean and maximum values.

4.5 Spatiotemporal evaluation of ecotoxicological risks in the Paraopeba River

Spatiotemporal ecotoxicological risk evaluations were limited to metals for which monitoring data, and, consequently, acute and chronic RQs presented less than 80% of censored data: Al, Fe, Mn and Zn.

Previously to the spatial evaluation of risks associated to the occurrence of Al, Fe, Mn and Zn in the monitored area, a Cluster Analysis (CA) was promoted. RQs from the four evaluated metals at the 25 stations monitored along the whole period were used as input simultaneously in the CA, for both acute and chronic effects. Groups which presented similar patterns risks formed clusters. According to Zhang *et al*. (2011) when data shows different dimensions it is necessary to standardize, in other words, to convert the database to Z scale (with mean and variance of zero and one, respectively) – by subtracting the mean from each observation and dividing by the standard deviation ($(X-\mu)/\sigma$). Since RQs are dimensionless, this step was not necessary in the analysis applied in this study.

CAs were performed in software Statistica® 10.0 based on Euclidean distance as a measure of similarity and on the Ward's Method of Agglomerative Hierarchical Clustering as a measure of distance between two clusters, which is determined based on the cluster's variance analysis (SHRESTHA; KAZAMA, 2007; ZHANG *et al.*, 2011). The clusters division line was defined by visual inspection. Groups were formed based on this division line as according to acute and chronic dendrograms.

In addition, the monitoring period from January 2019 to February 2022 was split as according to the hydrological year into wet (October to March) and dry (April to September) (FERREIRA *et al.*, 2021) for following periods: (i) wet period 2019 (W19); (ii) dry period 2019 (D19); (iii) wet period 2019-2020 (W19-20); (iv) dry period 2020 (D20); (v) wet period 2020-2021 (W20-21); (vi) dry period 2021 (D21); and (vii) wet period 2021-2022 (W21-22).

The spatial evolution of risks along the Paraopeba River was evaluated by comparing the defined regions based on results obtained from the CA, within periods of hydrologic year defined between 2019 and 2022. Differences between defined regions within each hydrological period were evaluated by the nonparametric Kruskal-Wallis test, followed by Dunn's multiple comparison test (when applicable) (5% of significance level). These tests were used based on previous analyses of data distribution by the Shapiro-Wilk normality test (α = 5%).

For the evaluation of temporal evolution of acute and chronic ecotoxicological risks in the water of the Paraopeba River, time series were constructed for each defined region (based on results and considerations of CA) and metal (Al, Fe, Mn and Zn) considering observations made from January 26th, 2019 to February 14th, 2022. Time series were used to evaluate the occurrence of seasonal changes, patterns and profiles on ecological risks to aquatic fauna in the Paraopeba River water since the B1 dam collapse.

5 RESULTS AND DISCUSSION

5.1 Paraopeba River water quality database description

Table 5 presents a descriptive analysis of data corresponding to the 14 dissolved metals and metalloids monitored in each of the 25 monitoring points along the Paraopeba River as according to the following parameters: number of observations, median, percentage of censored data and percentage violation of legal limits (BRASIL, 2005; MINAS GERAIS, 2022).

Dissolved Ag was the metal for which a lower number of observations were available (7,087), as this metal was only monitored in all monitoring points until January 2021. After that, analysis of this metal was limited to PT-01 and PT-09, both at Brumadinho city. Furthermore, all data obtained for this metal were censored as values obtained for all observations were below the limit of quantification. This corroborates with the study developed by Vergilio *et al*., (2020) who analyzed the Paraopeba River water five days after B1 dam tailings collapse and observed that Ag values were below LOQ in all samples and below the CONAMA Resolution n° 357/05 (BRASIL, 2005).

Besides Ag, dissolved As, Cd, Co, Cr, Cu, Hg, Ni, Pb and U presented, in general, more than 80% of censored data and less than 1% of violation of legal limits. A study conducted in two monitoring campaigns (October 2019 and March 2020) along 10 points of the Paraopeba River also did not find any violations of CONAMA Resolution n° 357/05 for As, Cr, Cu and Pb (TERAMOTO *et al*., 2021). However, not violating legal limits does not mean that river water cannot be toxic to aquatic organisms.

Table 5 – Descriptive analysis of observations registered for dissolved metals and metalloids in the Paraopeba River water quality database used in this study

Monitoring point	Ag				Al				As				Cd			
	No. obs.*	Median (mg L⁻¹)	% censored data	% legal violation	No. obs.*	Median (mg L⁻¹)	% censored data	% legal violation	No. obs.*	Median (mg L⁻¹)	% censored data	% legal violation	No. obs.*	Median	% censored data	% legal violation
PT-52	244	0.001	100.0%	0.0%	934	0.120	2.0%	56.0%	934	0.001	99.5%	0.0%	934	0.001	100.0%	0.0%
PT-01	479	0.005	100.0%	0.0%	875	0.120	5.3%	55.3%	876	0.001	94.3%	0.0%	876	0.001	100.0%	0.0%
PT-13	286	0.005	100.0%	0.0%	724	0.110	5.8%	51.2%	724	0.001	98.9%	0.0%	724	0.001	100.0%	0.0%
PT-09	406	0.005	100.0%	0.0%	1,078	0.120	5.6%	55.3%	1,069	0.001	96.4%	0.0%	1,077	0.001	99.9%	0.0%
PT-02	291	0.005	100.0%	0.0%	964	0.130	3.9%	60.7%	965	0.001	96.0%	0.0%	965	0.001	100.0%	0.0%
PT-05	274	0.003	100.0%	0.0%	952	0.140	6.2%	61.8%	953	0.001	91.8%	0.0%	953	0.001	99.9%	0.0%
PT-03	276	0.003	100.0%	0.0%	554	0.126	12.1%	55.8%	554	0.001	85.4%	0.0%	554	0.001	99.8%	0.0%
PT-14	272	0.001	100.0%	0.0%	612	0.094	5.6%	47.4%	612	0.001	99.7%	0.0%	612	0.001	100.0%	0.0%
PT-48	265	0.003	100.0%	0.0%	576	0.139	11.3%	58.2%	576	0.001	80.0%	0.2%	576	0.001	99.8%	0.0%
PT-04	267	0.003	100.0%	0.0%	523	0.130	13.0%	57.0%	523	0.001	78.2%	0.0%	523	0.001	100.0%	0.0%
PT-15	276	0.003	100.0%	0.0%	669	0.167	8.7%	66.8%	669	0.001	81.6%	0.1%	669	0.001	99.9%	0.1%
PT-53	243	0.001	100.0%	0.0%	520	0.080	4.6%	43.3%	520	0.001	96.7%	0.0%	520	0.001	100.0%	0.0%
PT-54	242	0.001	100.0%	0.0%	504	0.103	2.8%	50.4%	504	0.001	96.2%	0.2%	504	0.001	100.0%	0.0%
PT-43	272	0.001	100.0%	0.0%	572	0.100	2.8%	49.7%	572	0.001	97.2%	0.2%	572	0.001	100.0%	0.0%
PT-16	275	0.001	100.0%	0.0%	552	0.104	2.5%	51.3%	552	0.001	97.1%	0.0%	552	0.001	100.0%	0.0%
PT-17	273	0.001	100.0%	0.0%	555	0.091	2.7%	47.7%	555	0.001	97.8%	0.0%	555	0.001	100.0%	0.0%
PT-18	272	0.001	100.0%	0.0%	547	0.102	2.7%	50.8%	546	0.001	97.4%	0.2%	547	0.001	100.0%	0.0%
PT-49	245	0.001	100.0%	0.0%	591	0.110	1.4%	52.8%	591	0.001	97.0%	0.0%	591	0.001	100.0%	0.0%
PT-19	298	0.005	100.0%	0.0%	619	0.100	7.1%	47.3%	619	0.001	98.5%	0.0%	619	0.001	100.0%	0.0%
PT-55	245	0.001	100.0%	0.0%	695	0.116	3.0%	54.0%	697	0.001	97.8%	0.0%	697	0.001	100.0%	0.0%
PT-20	297	0.005	100.0%	0.0%	752	0.100	22.2%	48.4%	751	0.001	98.4%	0.0%	752	0.001	100.0%	0.0%
PT-28	270	0.001	100.0%	0.0%	923	0.054	22.6%	42.5%	923	0.001	99.0%	0.0%	923	0.001	100.0%	0.0%
PT-21	274	0.001	100.0%	0.0%	925	0.048	28.6%	39.9%	925	0.001	99.7%	0.0%	925	0.001	100.0%	0.0%
CE-02	247	0.001	100.0%	0.0%	863	0.059	29.5%	43.3%	863	0.001	100.0%	0.0%	863	0.001	100.0%	0.0%
PT-22	298	0.005	100.0%	0.0%	633	0.040	36.7%	30.8%	633	0.001	100.0%	0.0%	633	0.001	100.0%	0.0%

*Total number of observations

** Censored data: below the limit of quantification for each method

(Continues)

Table 5 (sequence) – Descriptive analysis of observations registered for dissolved metals and metalloids in the Paraopeba River water quality database used in this study

Monitoring point	Co				Cr				Cu				Fe			
	No. obs.*	Median	% censored data	% legal violation	No. obs.*	Median	% censored data	% legal violation	No. obs.*	Median	% censored data	% legal violation	No. obs.*	Median	% censored data	% legal violation
PT-52	797	0.001	100.0%	0.0%	934	0.001	98.8%	0.0%	927	0.001	82,4%	0.0%	934	0.380	0.0%	63.8%
PT-01	605	0.010	92.9%	0.0%	876	0.010	100.0%	0.0%	876	0.007	86,1%	0.2%	874	0.485	0.0%	71.1%
PT-13	474	0.010	98.5%	0.0%	724	0.010	100.0%	0.0%	724	0.007	94,1%	0.0%	724	0.430	0.0%	67.0%
PT-09	936	0.010	96.3%	0.0%	1,078	0.010	99.7%	0.0%	1,076	0.007	89,6%	0.7%	1,078	0.430	0.0%	70.9%
PT-02	828	0.010	94.3%	0.0%	965	0.010	100.0%	0.0%	963	0.007	87,7%	0.9%	965	0.500	0.0%	78.9%
PT-05	816	0.001	90.6%	0.0%	953	0.005	99.4%	0.0%	945	0.001	70,6%	0.8%	953	0.419	0.0%	69.4%
PT-03	554	0.001	86.5%	0.0%	554	0.005	99.3%	0.0%	554	0.001	82,0%	0.7%	554	0.346	0.0%	57.4%
PT-14	612	0.001	99.3%	0.0%	612	0.001	99.0%	0.0%	612	0.001	85,1%	0.0%	612	0.344	0.0%	55.1%
PT-48	576	0.001	86.5%	0.0%	576	0.005	99.1%	0.0%	576	0.001	65,0%	0.7%	576	0.352	0.0%	57.6%
PT-04	523	0.001	83.6%	0.0%	523	0.005	98.7%	0.0%	523	0.001	62,0%	1.1%	523	0.320	0.0%	53.5%
PT-15	669	0.001	88.8%	0.0%	669	0.005	99.3%	0.0%	669	0.001	60,1%	0.1%	669	0.380	0.0%	60.7%
PT-53	520	0.001	99.4%	0.0%	520	0.001	98.3%	0.0%	520	0.001	73,5%	0.0%	520	0.289	0.0%	48.1%
PT-54	504	0.001	99.4%	0.0%	504	0.001	98.2%	0.0%	504	0.001	70,6%	0.0%	504	0.280	0.0%	47.2%
PT-43	572	0.001	99.8%	0.0%	572	0.001	97.9%	0.0%	572	0.001	75,5%	0.0%	572	0.282	0.0%	47.4%
PT-16	552	0.001	99.8%	0.0%	552	0.001	98.9%	0.0%	552	0.001	72,1%	0.0%	552	0.279	0.0%	45.8%
PT-17	555	0.001	100.0%	0.0%	555	0.001	98.2%	0.0%	555	0.001	81,0%	0.0%	554	0.254	0.0%	44.4%
PT-18	547	0.001	99.8%	0.0%	547	0.001	98.2%	0.0%	547	0.001	83,0%	0.0%	547	0.273	0.0%	45.9%
PT-49	591	0.001	99.8%	0.0%	591	0.001	98.8%	0.0%	591	0.001	70,1%	0.2%	591	0.278	0.0%	47.5%
PT-19	619	0.010	100.0%	0.0%	619	0.010	99.8%	0.0%	619	0.007	94,7%	0.5%	619	0.327	0.0%	53.2%
PT-55	697	0.001	100.0%	0.0%	697	0.001	99.0%	0.0%	695	0.001	73,2%	0.0%	696	0.310	0.0%	50.9%
PT-20	752	0.010	100.0%	0.0%	752	0.010	100.0%	0.0%	752	0.007	89,8%	0.0%	752	0.310	0.0%	50.5%
PT-28	923	0.001	99.8%	0.0%	923	0.001	98.9%	0.0%	920	0.001	86,8%	0.0%	923	0.128	0.0%	32.4%
PT-21	925	0.001	99.9%	0.0%	925	0.001	99.7%	0.0%	925	0.001	86,5%	0.1%	925	0.100	0.0%	27.8%
CE-02	863	0.001	99.9%	0.0%	863	0.001	99.2%	0.0%	862	0.001	86,7%	0.0%	863	0.126	0.0%	30.1%
PT-22	633	0.010	100.0%	0.0%	633	0.010	99.8%	0.0%	633	0.007	99,1%	0.0%	633	0.100	0.0%	28.3%

*Total number of observations
** Censored data: below the limit of quantification for each method

(Continues)

Table 5 (sequence) – Descriptive analysis of observations registered for dissolved metals and metalloids in the Paraopeba River water quality database used in this study

Monitoring point	Hg				Mn				Ni				Pb			
	No. obs.*	Median	% censored data	% legal violation	No. obs.*	Median	% of censored data	% legal violation	No. obs.*	Median	% censored data	% legal violation	No. obs.*	Median	% censored data	% legal violation
PT-52	934	0.0001	100.0%	0.0%	883	0.020	26.4%	1.7%	934	0.001	98.5%	0.0%	934	0.001	98.3%	0.0%
PT-01	865	0.0002	100.0%	0.0%	825	0.030	25.7%	6.8%	876	0.010	99.8%	0.0%	875	0.010	99.3%	0.0%
PT-13	724	0.0002	99.9%	0.1%	672	0.030	14.4%	3.7%	724	0.010	100.0%	0.0%	723	0.010	99.3%	0.0%
PT-09	1,059	0.0002	100.0%	0.0%	1,010	0.030	16.8%	7.3%	1,078	0.010	99.4%	0.0%	1,078	0.010	98.6%	0.3%
PT-02	954	0.0002	100.0%	0.0%	912	0.036	14.0%	7.2%	965	0.010	99.3%	0.1%	965	0.010	99.0%	0.2%
PT-05	944	0.0001	100.0%	0.0%	901	0.041	7.8%	11.9%	953	0.005	98.2%	0.0%	953	0.002	98.0%	0.4%
PT-03	543	0.0001	100.0%	0.0%	502	0.077	1.6%	36.3%	554	0.005	97.8%	0.0%	554	0.005	96.9%	0.4%
PT-14	612	0.0001	100.0%	0.0%	560	0.050	7.5%	16.3%	612	0.001	98.9%	0.0%	605	0.001	98.7%	0.0%
PT-48	576	0.0001	100.0%	0.0%	523	0.095	2.7%	47.2%	576	0.005	96.7%	0.0%	576	0.005	97.9%	0.3%
PT-04	513	0.0001	99.8%	0.0%	470	0.111	1.7%	55.3%	523	0.005	96.9%	0.0%	523	0.005	97.3%	0.6%
PT-15	660	0.0001	100.0%	0.0%	614	0.080	5.5%	42.7%	669	0.005	98.4%	0.0%	668	0.002	97.0%	0.1%
PT-53	520	0.0001	100.0%	0.0%	469	0.056	3.0%	29.0%	520	0.001	97.3%	0.0%	520	0.001	98.8%	0.0%
PT-54	504	0.0001	100.0%	0.0%	452	0.045	3.3%	8.2%	504	0.001	97.6%	0.0%	504	0.001	99.6%	0.0%
PT-43	572	0.0001	100.0%	0.0%	520	0.014	14.4%	2.1%	572	0.001	98.3%	0.0%	565	0.001	98.8%	0.0%
PT-16	552	0.0001	100.0%	0.0%	500	0.012	11.6%	2.8%	552	0.001	97.1%	0.0%	542	0.001	99.1%	0.0%
PT-17	555	0.0001	100.0%	0.0%	502	0.020	6.8%	2.2%	555	0.001	98.6%	0.0%	545	0.001	99.8%	0.0%
PT-18	547	0.0001	100.0%	0.0%	495	0.015	8.7%	2.0%	547	0.001	98.0%	0.0%	540	0.001	99.3%	0.0%
PT-49	591	0.0001	100.0%	0.0%	539	0.009	25.2%	1.3%	591	0.001	98.8%	0.0%	591	0.001	99.0%	0.2%
PT-19	619	0.0002	100.0%	0.0%	567	0.020	56.4%	1.6%	619	0.010	99.5%	0.0%	610	0.010	99.7%	0.0%
PT-55	697	0.0001	100.0%	0.0%	643	0.014	34.7%	1.4%	697	0.001	98.9%	0.0%	695	0.001	99.0%	0.0%
PT-20	751	0.0002	100.0%	0.0%	701	0.020	60.8%	3.4%	752	0.010	99.9%	0.0%	743	0.010	99.3%	0.0%
PT-28	923	0.0001	100.0%	0.0%	868	0.004	55.9%	0.7%	923	0.001	98.9%	0.0%	918	0.001	99.8%	0.1%
PT-21	925	0.0001	100.0%	0.0%	872	0.003	53.6%	0.6%	925	0.001	98.5%	0.0%	915	0.001	99.8%	0.0%
CE-02	863	0.0001	100.0%	0.0%	812	0.006	38.7%	1.8%	863	0.001	99.0%	0.0%	863	0.001	99.9%	0.0%
PT-22	633	0.0002	100.0%	0.0%	581	0.020	66.1%	1.7%	633	0.010	99.7%	0.0%	623	0.010	100.0%	0.0%

*Total number of observations
** Censored data: below the limit of quantification for each method

(Continues)

Table 5 (sequence) – Descriptive analysis of observations registered for dissolved metals and metalloids in the Paraopeba River water quality database used in this study

Monitoring point	U				Zn			
	No. obs.*	Median	% censored data**	% legal violation	No. obs.*	Median	% censored data**	% legal violation
PT-52	934	0.001	99.8%	0.0%	934	0.004	80.6%	0.1%
PT-01	874	0.001	99.8%	0.0%	876	0.020	90.1%	0.1%
PT-13	723	0.001	99.7%	0.0%	724	0.020	97.0%	0.0%
PT-09	1,069	0.001	99.4%	0.0%	1,078	0.020	93.1%	0.0%
PT-02	964	0.001	99.7%	0.0%	965	0.020	91.8%	0.0%
PT-05	953	0.001	99.6%	0.0%	953	0.007	76.8%	0.1%
PT-03	554	0.005	99.6%	0.0%	554	0.005	61.7%	0.0%
PT-14	605	0.001	99.7%	0.0%	612	0.001	74.7%	0.0%
PT-48	576	0.001	100.0%	0.0%	576	0.005	57.8%	0.0%
PT-04	523	0.005	99.8%	0.0%	523	0.005	55.1%	0.0%
PT-15	669	0.001	100.0%	0.0%	668	0.007	63.8%	0.0%
PT-53	520	0.001	100.0%	0.0%	520	0.001	70.2%	0.0%
PT-54	504	0.001	100.0%	0.0%	504	0.001	68.7%	0.2%
PT-43	565	0.001	99.8%	0.0%	572	0.001	71.7%	0.0%
PT-16	542	0.001	100.0%	0.0%	552	0.001	72.5%	0.0%
PT-17	547	0.001	100.0%	0.0%	553	0.001	71.1%	0.0%
PT-18	540	0.001	100.0%	0.0%	547	0.001	72.2%	0.2%
PT-49	591	0.001	100.0%	0.0%	591	0.001	75.0%	0.0%
PT-19	610	0.001	100.0%	0.0%	619	0.020	94.5%	0.0%
PT-55	697	0.001	100.0%	0.0%	697	0.002	77.2%	0.0%
PT-20	742	0.001	100.0%	0.0%	752	0.020	95.5%	0.0%
PT-28	918	0.001	99.9%	0.0%	923	0.002	74.1%	0.1%
PT-21	916	0.001	100.0%	0.0%	925	0.002	74.9%	0.3%
CE-02	863	0.001	100.0%	0.0%	863	0.002	71.3%	0.0%
PT-22	623	0.001	100.0%	0.0%	633	0.020	96.5%	0.0%

*Total number of observations
** Censored data: below the limit of quantification for each method

Source: author (2023)

Amongst all 14 metals and metalloids evaluated in the PMQAS database (Table 5), dissolved Al, Fe and Mn were the ones which presented the highest amount of violation of legal limits (BRASIL, 2005; MINAS GERAIS, 2022) in most monitoring stations, as it can be observed in Table 5. This is not surprising as these were the main metals present in tailings released from the B1 dam (TERAMOTO *et al.*, 2021; VERGILIO *et al.*, 2020). It is important to mention that the Paraopeba River has a history of anthropic contamination, with detach for mining activities (SOARES; PINTO; OLIVEIRA, 2020), and the Quadrilátero Ferrífero region – where the study area is located – is intrinsically rich in Fe, Al and Mn (PORSANI; DE JESUS; STANGARI, 2019), which justifies the legal limits violation for these metals even in the PT-52, in the upstream area. Besides highest amount of violation of legal limits, Al, Fe, Mn, and Zn were the metals which presented, in average, less than 80% of censored data.

For Al, highest medians were observed in monitoring points PT-05 (0.140 mg L^{-1}), PT-48 (0.139 mg L^{-1}) and PT-15 (0.167 mg L^{-1}), located, respectively, 11.7, 48.3 and 55 km downstream the B1 dam (at cities of Mario Campos, Betim and Juatuba, respectively). These medians were about 1.5 times higher than the Brazilian and Minas Gerais state legislation standards (0.100 mg L^{-1}) (BRASIL, 2005; MINAS GERAIS, 2022). Regarding Fe, PT-01 (0.485 mg L^{-1}), located 0.4 km upstream the B1 dam collapse point, and PT-13 (0.430 mg L^{-1}), PT-09 (0.430 mg L^{-1}) and PT-02 (0.500 mg L^{-1}), respectively located 0.2, 5.6 and 10.3 km downstream the B1 dam (all of them in Brumadinho city), presented the highest medians which were, approximately, 1.5 times higher than the legal limits (0.300 mg L^{-1}). For Mn, highest median concentrations were observed at 48.3 and 51.6 km downstream the B1 dam (Betim and Juatuba), respectively in PT-48 (0.095 mg L^{-1}) and PT-04 (0.111 mg L^{-1}) and were close to the legal limits (0.100 mg L^{-1}). For the other metals and metalloids, medians were, generally, equal to the LOQ of the analytical method.

Maximum concentrations of metals and metalloids were generally observed in monitoring points located less than 20 km downstream the Ferro Carvão confluence with the Paraopeba River, less than one month after the B1 dam collapse (Table 6). PT-02, located about 10 km downstream the dam (Brumadinho city), was the station where maximum concentrations were most frequently observed (for Co, Cu, Fe, Pb and Ni), followed by PT-05 (Mario Campos city), where Mn and U maximum concentrations were observed, in January 31st, 2019. Maximum concentrations of Cr (0.0278 mg L^{-1}) and Zn (0.5650 mg L^{-1}) were observed, respectively, 89.5 and 309 km downstream the former dam area after September 2019. These concentrations were above maximum concentrations reported by IGAM in the historical evaluation of the Paraopeba River, between 2000 and 2018 (0.06 and 0.43 mg L^{-1}, respectively for Cr and Zn) (IGAM, 2019b), thus indicating possible influence of the event.

Regarding As and Cd, maximum concentrations (0.0224 and 0.0011 mg L^{-1}, respectively) were observed more than 55 km downstream the collapse point and more than eight months after, which does not indicate a clear correlation between maximum concentrations of these metals and the dam rupture. Furthermore, maximum concentrations detected in the database used this study were similar to maximum values reported by IGAM, between 2000 and 2018: As – 0.0214 mg L^{-1}; Cd – 0.0014 mg L^{-1}, both at Mario Campos City, about 25 km downstream the collapse point (IGAM, 2019b).

Table 6 – Maximum concentration of metals and metalloids observed in the Paraopeba River after the B1 dam collapse and details corresponding to maximum values

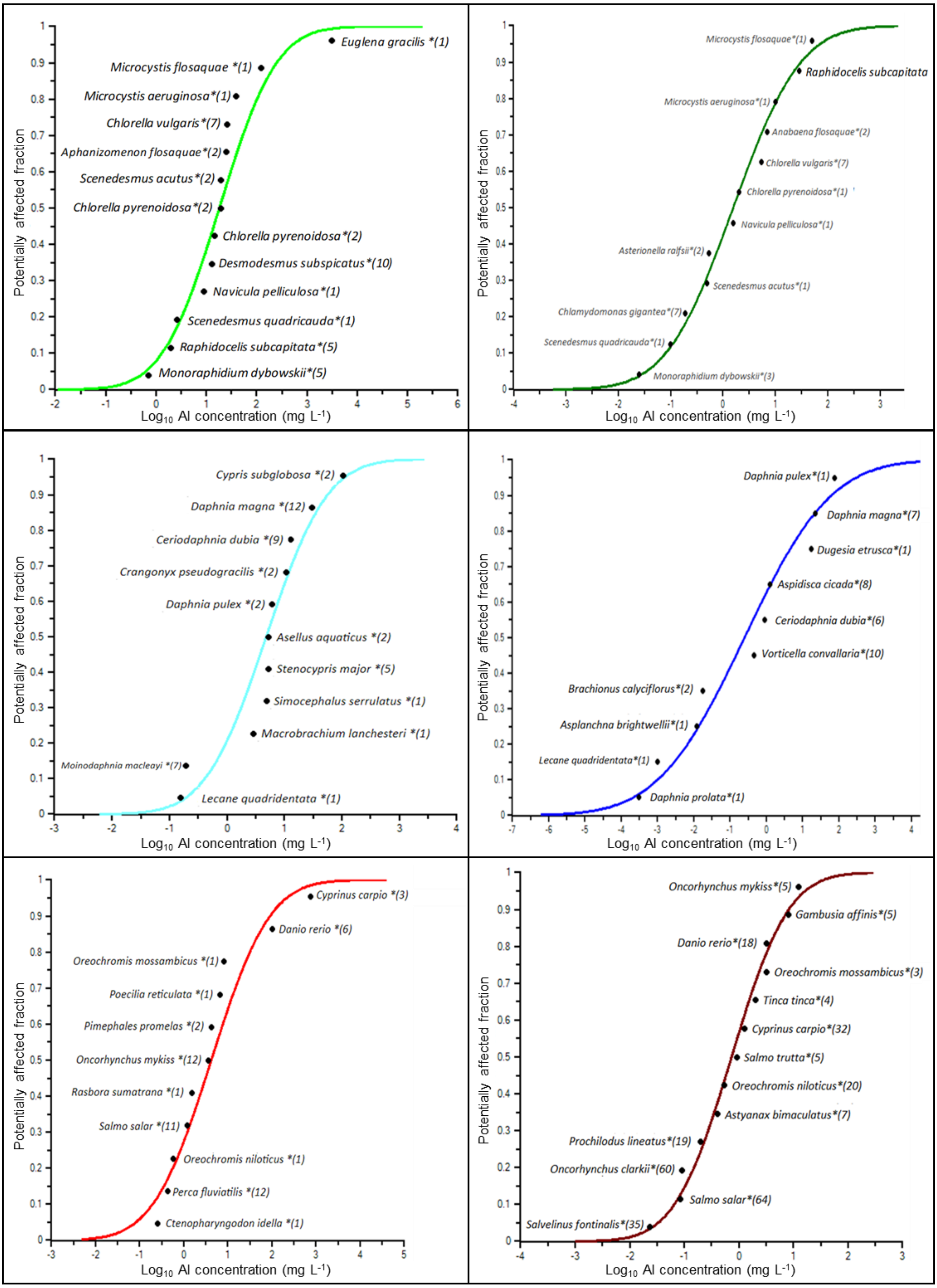
Metal	Maximum (mg L^{-1})	Details corresponding to maximum observed value				
		Monitoring Station	Latitude	Longitude	City	Date
Al	7.2300	PT-09	20°8'27.1"S	44°12'2.7"W	Brumadinho	19/02/2019
As	0.0224	PT-43	19°42'33.7" S	44°29'54.1"W	Pará de Minas	08/09/2020
Cd	0.0011	PT-15	19°56'15.1"S	44°20' 13.4"W	Juatuba	11/11/2019
Pb	0.0708	PT-02	20°8'7.2"S	44°12'47.0 "W	Brumadinho	19/02/2019
Co	0.0233	PT-02	20°8'7.2"S	44°12'47.0 "W	Brumadinho	31/01/2019
Cu	0.0942	PT-02	20°8'7.2"S	44°12'47.0 "W	Brumadinho	22/02/2019
Cr	0.0278	PT-28	18°55'59.9"S	44°46'47.3" W	Pompéu	19/09/2019
Fe	50.000	PT-02	20°8'7.2"S	44°12'47.0 "W	Brumadinho	30/01/2019
Mn	7.7000	PT-05	20°4'42.5"S	44° 12' 31.0"W	Mario Campos	31/01/2019
Hg	0.0003	PT-13	20°9'27.0"S	44°9' 39.9"W	Brumadinho	03/07/2020
Ni	0.0594	PT-02	20°8'7.2"S	44°12'47.0 "W	Brumadinho	22/02/2019
U	0.0079	PT-05	20°4'42.5"S	44° 12' 31.0"W	Mario Campos	31/01/2019
Zn	0.5650	PT-54	19°46'58.2"S	44°25'28.5"W	Esmeraldas	02/12/2019

Source: author (2023)

5.2 Species Sensitivity Distribution and legal limits

Figure 6 presents SSD curves obtained for acute and chronic effects of Al to each of the three trophic levels evaluated, represented by algae, invertebrates and fishes. The SSD curves for the other 13 metals and metalloids are presented in Appendix A to M.

Figure 6 – SSD curves obtained for acute (left) and chronic (right) toxicity of Al: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)

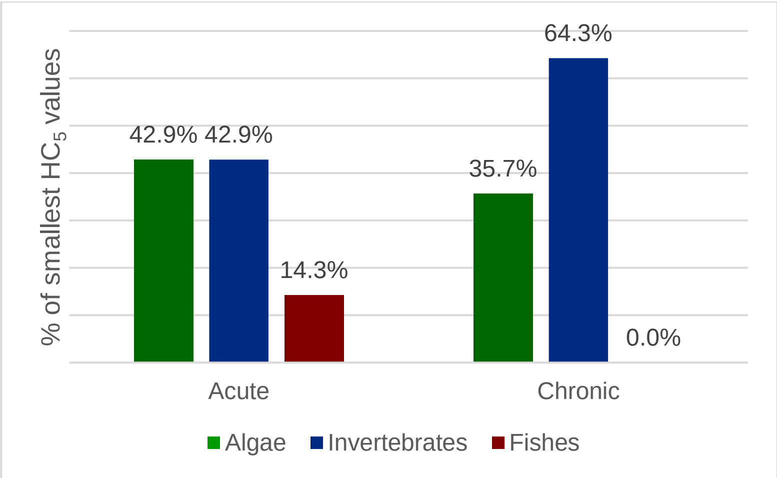


*(X) Number of observations used to plot the SSD curve for each species.

Source: author (2023)

Considering all the evaluated metals and metalloids, based on SSD curves, the smallest acute HC_5 values were defined by algae and invertebrates in 42.9% of compounds each and by fish in 14.3% (Figure 7). Regarding chronic HC_5, invertebrates defined the smallest HC_5 values for 64.3% metals and metalloids, while algae, defined for 35.7%. Fishes did not define chronic HC_5 values. Table 7 presents the most sensitive group for each metal/metalloid, considering acute and chronic effect, as well as the most sensitive specie for each group. These results reflect the higher sensitivity of algae and invertebrate to metals, indicating that ecotoxicological assays performed with these groups should be prioritized for events involving contamination by metals.

Figure 7 – Percentage of smallest acute and chronic HC_5 values defined by each group of organisms for all the evaluated metals and metalloids



Source: author (2023)

Table 7 – Acute and chronic most sensitive group and the corresponding most sensitive specie per metal and metalloid, based on HC_5 values

Metal	Acute		Chronic	
	Most sensitive group	Most sensitive specie	Most sensitive group	Most sensitive specie
Ag	Algae	*Ochromonas danica*	Invertebrates	*Daphnia galeata*
Al	Fishes	*Ctenopharygodon idella*	Invertebrates	*Daphnia prolata*
As	Fishes	*Coregonus hayi*	Algae	*Raphidocellis subcapitata*
Cd	Algae	*Melosira granulata*	Algae	*Parachlorella kessleri*
Co	Algae	*Scenedesmus acuminatus*	Invertebrates	*Orconectes hylas*
Cr	Algae	*Stephanodiscus hantzschii*	Algae	*Staurastrum cristatum*
Cu	Invertebrates	*Blepharisma americanum*	Invertebrates	*Lecane luna*
Fe	Algae	*Ankistrodesmus falcatus*	Invertebrates	*Daphnia prolata*
Hg	Invertebrates	*Daphnia carinata*	Invertebrates	*Hyalella curvispina*
Mn	Algae	*Chlorella vulgaris*	Algae	*
Ni	Invertebrates	*Colpidium colpoda*	Invertebrates	*Orconectes hylas*
Pb	Invertebrates	*Macrobrachium lanchesteri*	Invertebrates	*Astacus leptodactylus*
U	Invertebrates	*Ceriodaphnia dubia*	Invertebrates	*
Zn	Algae	*Melosira granulata*	Algae	*Microcystis aeruginosa*

*Not possible to define as smallest chronic value was obtained dividing acute HC_5 value by 10.

Source: Author (2023).

With respect to the construction of Species Sensitivity Distribution curves, it was challenging to reunite data from the literature regarding ecotoxicological assays, mainly for chronic effects. Data are generally scarce, related to different endpoints, based on a small group of standard species and assays are performed under standardized conditions (temperature, pH, DOC and hardness) which are not always presented in papers and also do not reflect natural settings. In addition, studies hardly report the source of metal they have used and whether they are reporting total or dissolved concentrations. Developing laboratory studies focused in obtaining data for SSD curves with different and native species of each trophic levels and under standardized conditions might be an alternative for these challenges.

Table 8 shows HC_5 values calculated through SSD curves for the 14 metals and metalloids and for each group representing the three trophic levels, compared to current Brazilian, Minas Gerais state and international standards. Considering that an AF of 1 was adopted in this study, PNEC values are equivalent to HC_5.

Table 8 – Acute and chronic HC_5 (PNEC) values obtained for dissolved metals and metalloids for algae, invertebrate and fishes in comparison to current Brazilian, Minas Gerais state and international standards

Metal	HC_5 (PNEC) (µg L^{-1})						Brazil and Minas Gerais (µg L^{-1}) [a]	Proposed PNEC (µg L^{-1}) WFD UK		EQSD (µg L^{-1}) EU [h]		NRWQC (µg L^{-1}) USA [i]		WQG (µg L^{-1}) Canada [j]	
	Acute			Chronic											
	A	I	F	A	I	F		Acute	Chronic	Maximum	Annual Average	Acute	Chronic	Acute	Chronic
Ag	**0.51**	**1.52**	**2.15**	0.47	**0.02**	**0.22**	10.0	nd	nd	nd	nd	3.2	nd	nd	0.25
Al	569.80	186.20	72.96	24.45	0.16	30.99	100.0	nd	nd	nd	nd	nd	nd	nd	100 [2]
As	54.36	370.60	11,960	7.02	37.06	1,200	10.0	8.0 [b]	0.5 [b]	nd	nd	340	150	nd	5
Cd	2.04	16.55	4.43	0.31	0.47	0.38	1.0	nd	nd	0.45 [1]	0.08	1.8	0.72	0.55 [2]	0.05 [2]
Co	52.05	1,430	714.40	1.86	0.29	65.48	50.0	nd	nd	nd	nd	nd	nd	nd	nd
Cr	16.76	15.65	5,208	2.85	6.39	23.88	50.0	2.0 [c]	3.4 [c]	nd	nd	16	11	nd	1 [2]
Cu	16.13	4.69	16.06	12.08	2.62	3.03	9.0	nd	8.2 [d]	nd	nd	nd	nd	nd	2 [2]
Fe	23.07	1,204	297.00	**0.28**	0.16	199.80	300.0	41.0 [e]	16.0 [e]	nd	nd	nd	1,000	nd	300
Hg	15.20	3.41	35.03	1.52	0.56	0.92	0.2	nd	nd	0.07	nd	1.4	0.77	nd	0.026
Mn	113.70	728.50	1,985	11.37	18.59	38.50	100.0	nd	123.0 [f]	nd	nd	nd	nd	2,081 [2]	380 [2]
Ni	20.60	6.64	6,192	3.46	0.68	17.42	25.0	nd	nd	34	4	470	52	nd	25 [2]
Pb	149.60	98.42	226.00	12.38	1.46	11.44	10.0	nd	nd	14	1.2	65	2.5	nd	1
U	-	25.15	500.60	-	2.52	17.09	20.0	nd	nd	nd	nd	nd	nd	33	15
Zn	5.50	98.11	267.00	0.87	3.63	14.39	180.0	nd	10.9 [g]	nd	nd	120	120	33.87 [2]	9.83 [2]

A = algae; I = invertebrates; F = fishes.

In **bold and underlined**: lowest value obtained for each group. In red: values smaller than the Brazil and Minas Gerais legal limits. nd: no data.

1 - Class 1 - hardness: < 40 mg L^{-1} $CaCO_3$; **2** - Hardness: 26.6 mg L^{-1}; pH: 7.22; DOC: 3 mg L^{-1}

a – CONAMA Resolution 357/05 (BRASIL, 2005) and Normative Deliberation COPAM/CERH n°08/22 (MINAS GERAIS, 2022)

b – United Kingdom Water Framework Directive – Proposed Environmental Quality Standard for Arsenic (LEPPER *et al.*, 2007)

c – United Kingdom Water Framework Directive – Proposed Environmental Quality Standard for Chromium (MAYCOCK *et al.*, 2007)

d – United Kingdom Water Framework Directive – Proposed Environmental Quality Standard for Cupper (COMBER; GEORGES, 2008)

e – United Kingdom Water Framework Directive – Proposed Environmental Quality Standard for Iron (JOHNSON *et al.*, 2007)

f – United Kingdom Water Framework Directive – Proposed Environmental Quality Standard for Manganese (PETERS *et al.*, 2010)

g – United Kingdom Water Framework Directive – Proposed Environmental Quality Standard for Zinc (MAYCOCK *et al.*, 2012)

h – European Union Environmental Quality Standards (EUROPEAN PARLIAMENT, 2013)

i – USA National Recommended Water Quality Criteria – Aquatic Life Criteria Table (US EPA, 2023)

j – Canadian Water Quality Guidelines for Aquatic Protection Life (CCME, 2022).

An evaluation of HC_5 values (Table 8) obtained in this study for all metals and metalloids indicates that Ag was the most toxic metal as it presented the smallest values for all trophic levels and effects. The only exception was observed for chronic effect in algae, for which Fe was the most toxic. For Ag acute effect, algae were the most sensitive group (HC_5 of 0.51 µg L^{-1}), while invertebrates were the most sensitive for chronic effects (HC_5 of 0.02 µg L^{-1}). Wang *et al*. (2019) observed an HC_5 of 0.88 µg L^{-1} for Ag acute effects in an SSD curve with 41 species of amphibians, invertebrates and fishes in the same SSD curve. Besides considering all species in the same curve, the results observed by the author is close to the acute HC_5 values identified in this study.

With respect to Al (Tables 7 and 8), fish was the most sensitive group for acute effects (HC_5 of 72.96 µg L^{-1}) while invertebrates were the most sensitive considering chronic effects (HC_5 of 0.16 µg L^{-1}). In an acute toxicity study with Al, Hui *et al*. (2016) also identified fish as the most sensitive group. Razak *et al*. (2021) and Gebara *et al*. (2020) obtained HC_5 values of, respectively, 521.06 µg L^{-1} and 148 µg L^{-1} for Al acute effects, which were close to the results observed in the present study for algae and invertebrates (569.8 and 186.2 µg L^{-1}, respectively). However, these authors considered the same SSD curve for all species together, and fish, which was the most sensitive group in the present study, would not be protected by the values observed by these researchers. This reflects the fact that SSD curves built with different trophic levels results in higher HC_5 values.

For Cd, algae were the most sensitive group for acute and chronic effects (HC_5 of 2.04 and 0.31 µg L^{-1}, respectively) (Tables 7 and 8). Algae were also the most sensitive group in a study conducted by Razak *et al*. (2021) which assessed Cd acute toxicity. Most studies on Cd toxicity considered different trophic levels in the same SSD curve. HC_5 acute values ranging from 3.1 to 23.8 µg L^{-1} were observed by Park and Kim (2020). Meanwhile, Arambawatta-Lekamge, Pathiratne and Rathnayake (2021) observed a chronic HC_5 value of 0.48 µg L^{-1} which is similar to the chronic values obtained in the present study.

Besides Cd, algae were also the most sensitive group for acute and chronic effects for Mn and Zn (Table 7). For Cu, Hg, Ni, Pb and U, invertebrates were the most sensitive group for both acute and chronic evaluations (Tables 7 and 8). For acute effects, similar results were observed by Lima *et al*. (2019), for Cu, Cd, and Hg, and by Razak *et al*. (2021) for Cu and Pb. Regarding chronic SSD curves, Arambawatta-Lekamge, Pathiratne and Rathnayake (2021) also verified invertebrates as the most sensitive group for Cu.

In relation to Co and Fe, invertebrates were the most sensitive group for chronic effects, with respective HC_5 of 0.29 and 0.16 µg L^{-1}, while these metals were more toxic for algae, regarding acute effects. For Mn, algae and fishes were, respectively, the most and least sensitive groups for both acute and chronic effect. These results are in accordance with the observed by Alho and collaborators (2022). Marks *et al*. (2017) also verified fish as the least sensitive group for chronic effects of Mn.

It was not possible to calculate HC_5 nor PNEC values for acute and chronic effects in algae for U due to absence of data in the literature. This calls attention to the need of studies which carry out algae exposure assays to this metal. This is probably a consequence of exposure risks associated to U manipulation in the laboratory. Considering the other trophic levels, HC_5 were lower for invertebrates (Table 7), thus being the most sensitive group to this metal.

It is important to note that results obtained through SSD curves and HC_5 calculations are highly dependent on the number of species; the proportion of each taxonomic group in the ecotoxicity database; the endpoint considered for each toxicity test (for example, EC_{50}, LC_{50}, NOEC, EC_{10}); the adopted distribution model of SSD curves (normal, logistic, triangular, etc.), amongst other factors (HAYASHI; KASHIWAGI, 2011). Thus, different studies may result in different HC_5 values for the same substance and the same toxic effect. For example, independent studies developed by Razak *et al*. (2021), Alho *et al*. (2022) and Harford *et al*. (2015), using the SSD methodology, observed different acute HC_5 values for Mn, which were, respectively: 1,049 µg L^{-1}, 580 µg L^{-1} and 143 µg L^{-1}. These are also different from results observed in the present study: 113.7 µg L^{-1} for algae, 728,5 µg L^{-1} for invertebrates and 1,985 µg L^{-1} for fish.

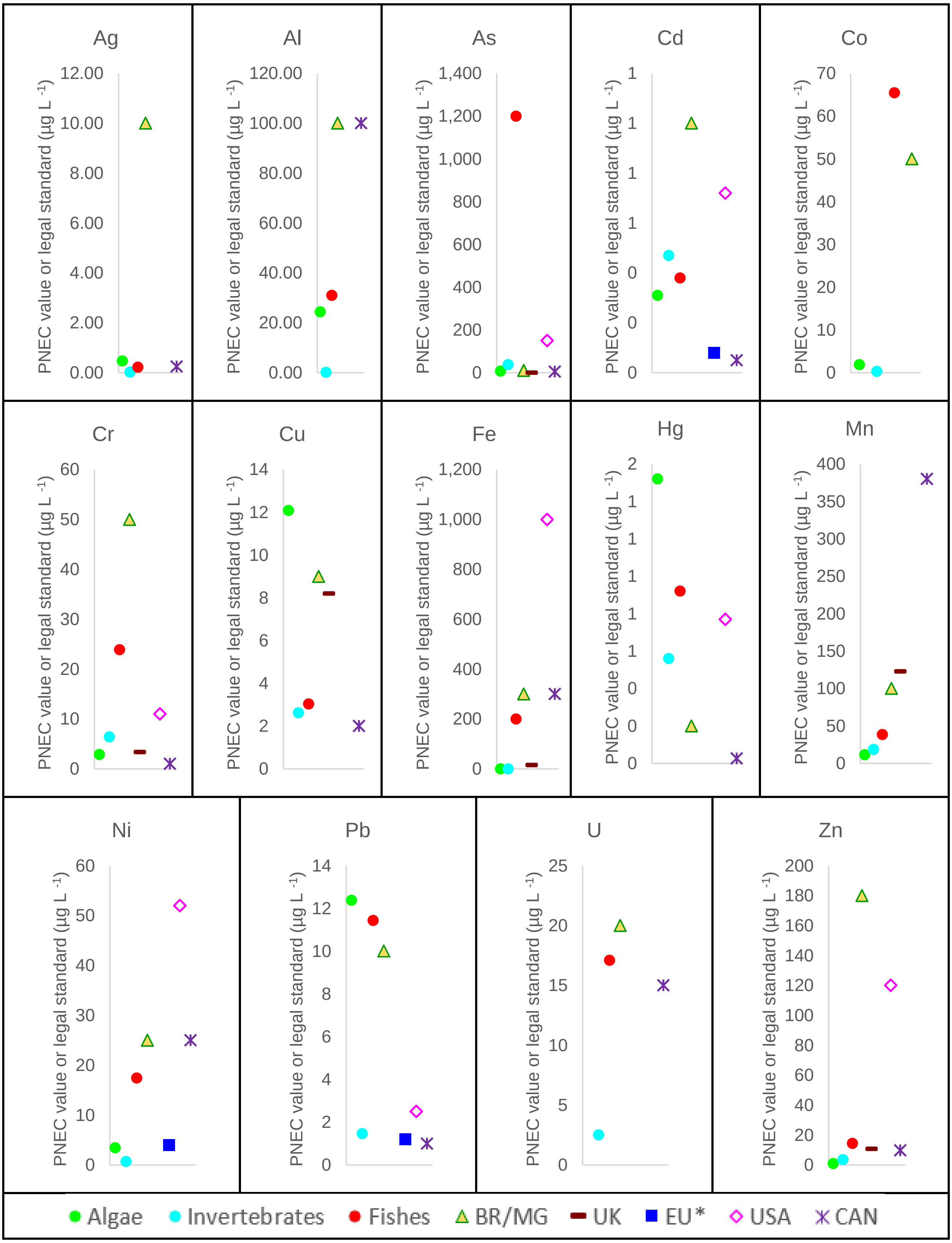
It is also important to highlight the acute and chronic values of HC_5 obtained via SSD curves built with organisms from different trophic levels in the same curve, as in results showed by Alho *et al*. (2022), Gebara *et al*. (2020), Lima et al. (2019), Razak *et al*. (2021) and by Wang, Kwok and Leung (2019) and HC_5 values derived from distinct SSD curves per trophic levels, as in the present study. Results of HC_5 obtained from separate curves per trophic level tend to be more conservative, present higher sensitivity and allow for a better identification of the most sensitive trophic level. However, this is the first time such curves are presented per trophic level for metals and metalloids in the scientific literature. Thus, the present study contributes to the evolution of ecotoxicology and environmental risk assessment as scientific tools.

5.2.1 Predicted No Effect Concentration and current legal limits

Regarding Brazilian and Minas Gerais state legal standards, Table 8 shows that 45 out of a total of 82 PNEC values obtained in this study were below current legal limits, mainly for chronic (78% of PNEC chronic values) when compared to acute effects (32% of PNEC acute values). In other words, according to PNEC values obtained via SSD curves built in this study, aquatic organisms might not be protected from toxic effects promoted by metals and metalloids by the current legislation in 54.9% of all cases and this is mainly true for chronic effects.

For Ag, Al, Cd, Cr, Fe, Mn, Ni, U and Zn, all PNEC values for chronic effects were below current legal limits (Figure 8), indicating that the evaluated groups might not be completely protected from chronic effects by the defined values in the Brazilian and Minas Gerais legislation. This is critical in the context of the B1 dam rupture as Al, Fe and Mn showed the highest percentages of violations ranging from 30.8 – 66.8%, 27.8 – 79.9%, 0.6 –55.3% for each of these elements, respectively (Table 5). On the other hand, all the calculated PNEC values corresponding to acute effects were higher than the current legal Brazilian and Minas Gerais standards for all evaluated groups for the following metals: As, Cd, Co, Hg, Mn, Pb and U (Figure 9).

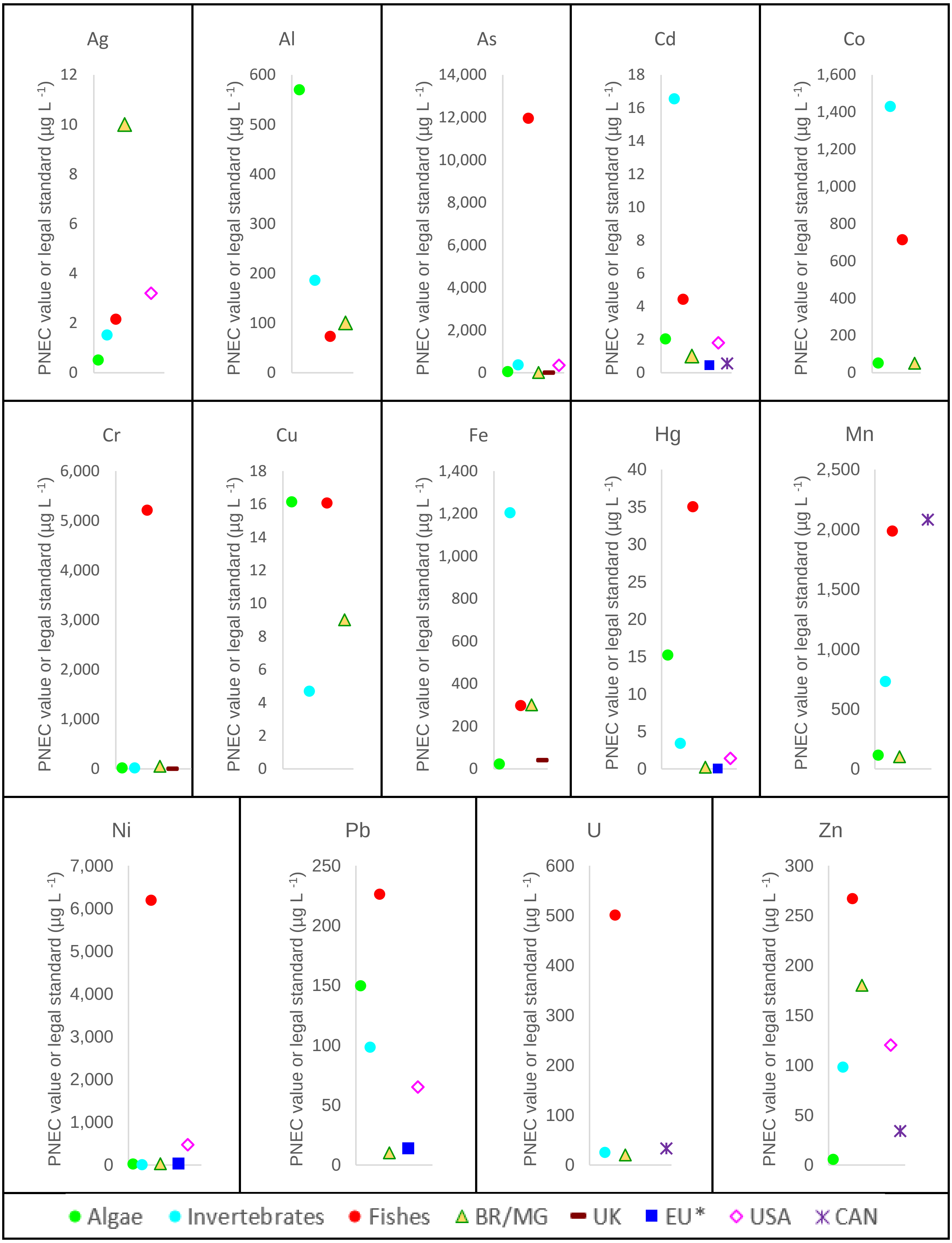
Figure 8 – Chronic PNEC values for dissolved metals and metalloids for algae, invertebrate and fishes in comparison to current Brazilian, Minas Gerais state and international standards



EU*: Chronic = Annual Average

Source: author (2023).

Figure 9 – Acute PNEC values for dissolved metals and metalloids for algae, invertebrate and fishes in comparison to current Brazilian, Minas Gerais state and international standards



EU*: Acute = Maximum Concentration
Source: author (2023).

Considering Ag, all current national/state (10 µg L^{-1}) and international standards used as reference in this study are higher than acute and chronic PNEC values obtained by SSD curves (Table 8, Figure 8 and Figure 9). Despite still below, PNEC values were close to aquatic life protections standards used in the USA (3.2 µg L^{-1}) and Canada (0.25 µg L^{-1}), respectively, for acute and chronic effects. Thus, indicating that worldwide environmental legislation does not protect 95% or more of the aquatic biota from chronic nor acute effects promoted by Ag, except for chronic effects in algae, which might be protected by the Canadian WQG.

For Al, Fe and Ni, the Brazilian and Minas Gerais legal standards are equal to the Canadian WQG (100, 300 and 25 µg L^{-1}, respectively). For these cases, these current standards may be protective of the aquatic biota for specific groups only for acute effects (Al: algae and invertebrates, Fe: invertebrates and Ni: fishes). In the ecological context, if the concentration of a metal reaches a legal limit that is not protective to the aquatic biota, the habitat function might be compromised, probably resulting in ecological imbalance.

With respect to As, current legal standards in Brazil and Minas Gerais (10 µg L^{-1}) are more likely to protect aquatic biota as they are below all PNEC values obtained for all evaluated groups (algae, invertebrates and fishes), except for chronic effects to algae (7.02 µg L^{-1}). In the international context, aquatic life protection criteria established for As in the UK (0.5 µg L^{-1}) and in Canada (5 µg L^{-1}) may hinder chronic effects to producers. For Cd, chronic PNEC values were below current Brazilian and Minas Gerais standards (1 µg L^{-1}), yet still above legal values set by the EU and Canadian criteria (0.08 and 0.05 µg L^{-1}, respectively) for chronic effects.

In relation to Pb (Table 8, Figure 8 and Figure 9), current legal standard in Brazil and Minas Gerais (10 µg L^{-1}) is smaller than PNEC values obtained for all evaluated groups (algae, invertebrates and fishes). The only exception is chronic effects to invertebrates, for which the PNEC was 1.46 µg L^{-1}. In other words, the legislation might be protective against chronic effects to these organisms. In contrast, PNEC values obtained for Pb were above aquatic life protection values in practice in Canada (1 µg L^{-1}).

Regarding Cr, PNEC values obtained by SSD curves were below current Brazilian legal limits (50 µg L^{-1}), except for acute effects to fishes. Considering chronic effects, national legal limits are not protective of any of the evaluated groups (Table 8 and Figure 8). This means that current national/state legislation does not protect against acute effect to producers nor primary consumers which is critical considering that the endpoint evaluated in acute toxicity bioassays is mainly death. In contrast, UK and Canadian guidelines are below PNEC values obtained for almost all groups and effects, thus being more protective to aquatic life.

For Hg, the current legal limit (0.2 µg L^{-1}) is smaller than all the PNEC values obtained in the present study, so Brazilian and Minas Gerais legislation might be protective for all trophic levels and effects (Table 8, Figure 8 and Figure 9). Furthermore, these PNEC values are, in general, higher than the European, Canadian and the USA water quality criteria. This may be a consequence of historical concerns related to environmental and human health hazards promoted by Hg contamination and bioaccumulation, such as the Minamata Bay case in Japan, where consuming of contaminated fishes with methylmercury resulted in people neurological disease due to this heavy metal poisoning (SHARMA *et al.*, 2021).

For Zn, only the PNEC for acute effects in fishes (267 µg L^{-1}) (Table 8 and Figure 9) was higher than the CONAMA Resolution n° 357/05 and COPAM/CERH n° 08/2022 standards (180 µg L^{-1}). For acute effects, Brazilian and Minas Gerais legal limits are nearly 33-fold higher than acute PNEC for algae and 1.8-fold higher than acute PNEC value for invertebrates. In relation to chronic effects (Figure 8), national and state legal limits are nearly 29-fold higher than the average PNEC, considering the three trophic levels. Gebara *et al*. (2020) also observed that current CONAMA n° 357/05 standards for Zn may not be protective to 95% of the species, since acute HC_5 obtained by this author for zinc in an SSD curve that encompassed fishes, invertebrates and algae was about 3-fold smaller than legal limits.

It is important to mention that an organism subjected to concentrations higher than the PNEC values or even the legal limits, will not necessarily suffer acute or chronic effects due to that exposal. The manifestation of effects also depends on biodynamics, exposure routes, metal accumulation capacity of the specie, time of exposure, feeding relationships, specie sensitivity, bioavailability, additive or synergistic toxic effects of mixtures of substances, amongst other factors (MEBANE *et al.*, 2020b; VÄÄNÄNEN *et al.*, 2018).

PNEC values obtained in the present study indicate a need to rethink and revise national and regional legal standards related to metals and metalloids based on the approach of aquatic life protection. This perspective is adopted in other countries, such as the USA and Canada, where different legal limits are adopted according to water use purpose and local water characteristics, instead of one legal limit based on the most restrictive water use, as it is currently adopted in Brazil and in Minas Gerais. Nevertheless, PNEC values obtained in this study should not be used to replace laboratory exposure assays, yet as guides of priority metals and groups to be selected for ecotoxicological studies performed in each region. Future data should consider the use of native species in laboratory assays, thus contributing to the modeling studies as well. Ag, Cr and Zn are the most critical metals considering the need to update legal standards, since PNEC values obtained in this study were much lower than current values.

The SSD curves built in the present study were developed considering single metal laboratory assays reported in the scientific literature, EnviroTox and ECOTOX database. However, in the environment, organisms are generally exposed to a mixture of metals and other substances that can increase or decrease the acute and chronic effects (VAN REGENMORTEL *et al.*, 2017). Constructing SSD curves for mixtures of metals can be an alternative for future studies, in order to allow for the definition of HC_5 and PNEC values which consider a mix of metals (NYS *et al.*, 2018).

It is important to mention that there was a recent update to Minas Gerais state legal standards in November 2022, when the Normative Deliberation COPAM/CERH n° 08/22 (MINAS GERAIS, 2022) replaced the Normative Deliberation COPAM/CERH n° 01/08 (MINAS GERAIS, 2008). However, quality guidelines for metals and metalloids were not updated in the occasion. Considering the relevance of mining activity in the state of Minas Gerais, recent occurrence of dam ruptures in the state, the number of dams located at Quadrilátero Ferrífero, and, particularity, the geology and soil composition of each of Brazilian states, it is fundamental to rethink and revise regional legal limits.

5.2.2 Bioavailability and HC_5 values

It is known that the bioavailability of metals and metalloids in the natural environment is influenced by physicochemical aspects such as pH, DOC and hardness as these factors affect metal/metalloid physical state, solubility, and speciation, thus, influencing the interaction between metals/metalloids and aquatic organisms and, consequently, their toxicity (EGOROVA; ANANIKOV, 2017). As toxicity assays used to define $L(E)C_{50}$, NOEC, EC_{10} and LOEC values used to construct acute and chronic SSD curves were all carried out under defined conditions of pH, hardness and organic content by following standard procedures defined for each test-organism, it is recommended to adapt HC_5 values to reflect bioavailability (HC_{5bio}) as according to local physicochemical conditions at the area under study. The correction of quality guidelines is currently performed in Europe (EUROPEAN PARLIAMENT, 2013), Canada (CCME, 2022) and the USA (US EPA, 2023) for Al, Cd, Cr, Cu, Mn, Ni and Zn so that legal standards and risk assessments are more realistic to environmental conditions in each area/watershed. In contrast, this adjustment is not performed to set environmental standards in Brazil nor in Minas Gerais.

Besides, most scientific studies on the assessment of ecotoxicological risks do not account for bioavailability as (i) this makes results less comparable to others and very specific to the area under study and (ii) it is restricted to a few elements for which there are freely available bioavailability software. In the current study, the adjustment of HC_5 into HC_{5bio} was conducted for Mn, Cu, Ni, Zn, Pb and Co, based on the existence of bioavailability conversion tools for these metals (BIO-MET, 2021; WFD, 2014).

Table 9 shows HC_5 and HC_{5bio} values, as well as the Bioavailability Factors (BioF) calculated by the software used for each metal (Co, Cu, Mn, Ni, Pb and Zn), based on physicochemical characteristics specific to the Paraopeba River after the tailings dam collapse (Jan/2019 to Feb/2022, median values of pH = 7.22; DOC = 3 mg L^{-1}; [Dissolved Ca] = 5.91 mg L^{-1}). It is important to emphasize that these results refer to the context of the Paraopeba River and different results may be observed if these metals were evaluated for other waterbodies or even for this same river in another period.

Table 9 – BioF, acute and chronic HC_5 and HC_{5bio} values obtained for metals for algae, invertebrate and fishes compared to national/state legal standards

Metal	BioF	BR/MG (μg L^{-1})[a]	Acute (μg L^{-1})						Chronic (μg L^{-1})					
			A		I		F		A		I		F	
			HC_5	HC_{5bio}	HC_5	HC_{5bio}	HC_5	HC_{5bio}	HC_5	HC_{5bio}	HC_5	HC_{5bio}	HC_5	HC_{5bio}
Co	**0.6**	**50.0**	52.05	86.75	1,430	2,383	714.40	1,191	1.86	3.11	0.29	0.49	65.48	109.13
Cu	**0.06**	**9.0**	16.13	268.83	4.69	78.17	16.06	267.67	12.08	201.33	2.62	43.58	3.03	50.42
Mn	**0.31**	**100.0**	113.70	366.77	728.50	2,350	1,985	6,403	11.37	36.68	18.59	59.97	38.50	124.19
Ni	**0.44**	**25.0**	20.60	46.82	6.64	15.08	6,192	14,073	3.46	7.87	0.68	1.56	17.42	39.59
Pb	**0.16**	**10.0**	149.60	935	98.42	615.13	226	1,413	12.38	77.38	1.46	9.15	11.44	71.50
Zn	**0.53**	**180.0**	5.50	10.37	98.11	185.11	267	503.77	0.87	1.64	3.63	6.84	14.39	27.15

HC_{5bio}: HC_5 adapted to account for bioavailability; A = algae; I = invertebrates; F = fishes. Water quality conditions used to calculate BioF: pH = 7.22; DOC = 3 mg L^{-1}; [Dissolved Ca] = 5.91 mg L^{-1}).
a – CONAMA Resolution n° 357/05 (BRASIL, 2005) and Normative Deliberation COPAM/CERH n°08/22 (MINAS GERAIS, 2022)

Source: author (2023)

As it can be observed from Table 9, when bioavailability was considered by applying the BioF, the concentration which causes effect to 5% of the aquatic organisms increased. As a consequence, HC_{5bio} are higher than HC_5. This occurs because the BioF considers the ratio of the metal that is able to interact with the test-organism and cause toxicity under defined conditions of pH, hardness and organic content. For Cu, for instance, when bioavailability was considered, the concentration that is prone to cause effect to 5% of species increased nearly 17-fold. This was the greatest rise observed amongst the evaluated metals. On the other hand, in the case of Co, when the bioavailability aspect was taken into consideration the HC_5 only increased by 1.7 times, the smallest observed result.

Regarding Ni, HC_{5bio} is 2.3-fold the HC_5 (Table 9) and the smallest value observed for this metal was related to chronic effects to invertebrates (1.56 µg Ni L^{-1}). The Annual Average value set by the European Union Water Framework Directive (4 µg Ni L^{-1}) is slightly higher than this one, as water quality conditions which are representative for the region lead to lower Ni bioavailability (pH 8.2, DOC = 2 mg L^{-1} and water harness = 40 mg L^{-1} of $CaCO_3$) (EUROPEAN PARLIAMENT, 2013). In contrast, Brazilian and Minas Gerais current standard (25 µg Ni L^{-1}) is still 16-fold higher than HC_{5bio} values obtained in this study even after accounting for bioavailability factors.

In the case of Mn, the HC_5 value increased by 3-fold after considering bioavailability. Amongst all legal standards evaluated in this study (Table 8), the Canadian legislation (CCME, 2022) is the only one that considers bioavailability to define water quality guidelines for this metal. According to their procedure, a modelling equation which considers pH and hardness is used to calculate the standard for each case. When representative values of pH seen in the Paraopeba River were used as input on the Canadian model for different conditions of hardness (25 and 670 mg L^{-1} of $CaCO_3$) the WQG for Mn varied from 380 to 1,100 µg Mn L^{-1}. When hardness was set to the median value observed in the Paraopeba during the evaluated period (26.6 mg L^{-1} of $CaCO_3$) and the pH varied from 5.8 to 8.4, the water quality guideline ranged between 290 to 200 µg Mn L^{-1}. Thus, for Mn, it was possible to observe that hardness has a direct influence on bioavailability, the opposite as pH effect.

Results obtained in this study emphasize the relevance and need to consider metal bioavailability when setting water quality standards and conducting risk assessment studies in Brazil, as such perspective is still incipient. Bioavailability might be the key to differences in toxicity effects observed in laboratory conditions compared to natural environment. After all, organisms are generally exposed to different conditions of water physicochemical characteristics, such as pH and hardness, in the environment compared to those conditions standardized for laboratory assays. So, it is important to consider how environmental conditions can influence metal solubility, complexation, oxidative state, and consequently on the bioavailability to define effective legal standards (EGOROVA; ANANIKOV, 2017).

5.3 Ecotoxicological risk assessment

The smallest PNEC values obtained for each of the 14 metals/metalloid in the first stage of this document were used in the ecotoxicological risk assessment carried out for Paraopeba River region affected by B1 dam rupture. Bioavailability was considered for Co, Cu, Mn, Ni, Pb and Zn by using the lowest HC_{5bio} obtained for each of these elements. Table 10 shows PNEC values used to calculate risks for each metal. In addition, it is important to mention that values pointed out as outliers were kept in the database since these values were predominant in dates corresponding to the tailings dam collapse or to seasonal variations.

Table 10 – PNEC values used for ecotoxicological risk assessment in this study

Metal	Acute	Chronic	Metal	Acute	Chronic
Ag	0.51	0.02	**Fe**	23.07	0.16
Al	72.96	0.16	**Hg**	3.41	0.56
As	54.36	7.02	**Mn***	366.77	36.68
Cd	2.04	0.31	**Ni***	15.08	1.56
Co*	86.75	0.49	**Pb***	615.13	9.15
Cr	15.65	2.85	**U**	25.15	2.52
Cu*	78.17	43.58	**Zn***	10.37	1.64

*Metals for which bioavailability was considered.

Source: author (2023)

Table 11 presents the descriptive analysis of acute and chronic Risk Quotients obtained for each of the 14 metals and metalloids. For Ag, since 100% of data were censored, it was not possible to indicate the monitoring point and date of the maximum RQ.

Considering the risk ranking proposed by Young, Chen and Yang (2021) (RQ < 0.1 – negligible risk; 0.1 ≤ RQ < 1 – low risk; 1 ≤ RQ < 10 moderate risk; RQ ≥ 10 high potential risk), through the colors presented in Table 11, it is possible to verify that 50% of metals presented high risks for at least one effect – acute or chronic. Al, Fe, Mn and Zn presented high risks for both acute and chronic effects. Acute risks for As, Cd, Pb and U were low, while chronic risks for these metals were medium. Hg was the metal that presented the smallest acute and chronic maximum risks, respectively classified as negligible and low.

The highest RQs for both acute (2,167) and chronic (314,465) effects were observed for Fe, in station PT-02, the third monitoring point downstream the B1 dam collapse (10.3 km) (Table 11). These RQs were observed five days after the event. This was expected since the tailings from the B1 dam were originated from the beneficiation of iron ore and, according to Vergilio *et al*. (2020), Fe was the main metal in the composition of the B1 dam tailings (264.9 mg g^{-1}).

Table 11 – Descriptive statistical analysis of acute and chronic Risk Quotients classified according to risk levels by considering maximum concentration observed for each in the database: green – negligible; blue – low; yellow – medium; red – high.

Metal	RQ type	25th percentile	Median	Mean	75th percentile	Maximum	Details corresponding to maximum observed value	
							Monitoring Station	Date
Ag	Acute	1.957	1.957	4.525	4.892	9.78	na	na
	Chronic	52.632	52.632	121.687	131.579	263.16		
Al	Acute	0.622	1.480	2.377	3.166	99.10	PT-09	19/02/2019
	Chronic	278	662	1,063	1,417	44,355		
As	Acute	0.018	0.018	0.018	0.018	0.41	PT-43	08/09/2020
	Chronic	0.142	0.142	0.142	0.142	3.19		
Cd	Acute	0.489	0.489	0.429	0.489	0.54	PT-15	11/11/2019
	Chronic	3.257	3.257	2.859	3.257	3.58		
Co	Acute	0.012	0.012	0.055	0.115	0.27	**PT-02**	31/01/2019
	Chronic	2.045	2.045	9.710	20.450	47.73		
Cr	Acute	0.064	0.319	0.344	0.639	1.78	PT-28	19/09/2019
	Chronic	0.351	1.756	1.893	3.512	9.77		
Cu	Acute	0.013	0.013	0.036	0.090	1.20	**PT-02**	22/02/2019
	Chronic	0.023	0.023	0.065	0.161	2.16		
Fe	Acute	6.502	14.001	16.762	22.887	2,167	**PT-02**	30/01/2019
	Chronic	943	2,031	2,432	3,320	314,465		
Hg	Acute	0.003	0.003	0.004	0.006	0.01	PT-13	03/07/2020
	Chronic	0.179	0.179	0.249	0.357	0.54		
Mn	Acute	0.040	0.055	0.138	0.133	20.99	PT-05	31/01/2019
	Chronic	0.404	0.545	1.378	1.333	209.94		
Ni	Acute	0.066	0.331	0.358	0.663	3.94	**PT-02**	22/02/2019
	Chronic	0.643	3.215	3.474	6.431	38.20		
Pb	Acute	0.002	0.003	0.007	0.016	0.12	**PT-02**	19/02/2019
	Chronic	0.109	0.219	0.449	1.093	7.74		
U	Acute	0.040	0.040	0.056	0.040	0.31	PT-05	31/01/2019
	Chronic	0.398	0.398	0.557	0.398	3.14		
Zn	Acute	0.096	1.003	1.195	1.928	54.47	PT-54	02/12/2019
	Chronic	0.611	6.353	7.573	12.217	345.14		

na: not applicable

Source: author (2023)

Maximum RQ values were also detected in PT-02 for acute and chronic effects for Co, Cu, Ni and Pb (Table 11). Maximum acute and chronic RQ values for Co (0.27 and 47.73, respectively) occurred six days after the dam collapse (31/01/2019). For the other cited elements, maximum RQ occurred about one month after the event. Considering these elements, only Cu and Pb were pointed out as possible indicators of tailings from B1 dam. Meanwhile, Co also presented high concentrations upstream the dam (VERGILIO *et al.*, 2020). Increased risks following six days (Co) or one month from the rupture (Cu, Ni and Pb) may be a consequence of rains or tailing movement close to the rupture site in order to rescue victims. According to FEAM, IEF and IGAM (2021), in the first quarter of 2020, a high volume of rains was registered in the Paraopeba River watershed, mainly during January and February, with volumes between 740 and 1,200 mm, while the normal volumes observed in the basin varies from 500 to 650 mm.

Al was the metal which presented the second highest RQ, reaching a maximum chronic risk of 44,355 and acute risk of 99.10, both within three weeks from the collapse (19/02/2019). These values were observed in PT-09, the second station downstream the confluence between the Paraopeba River and the Ferro-Carvão Stream, where the B1 dam collapsed (about 5.6 km downstream). Although Al was the second most present metal in the B1 dam tailings (10.8 mg g^{-1}) as observed by Vergilio *et al.*, (2020), it could not be considered by these authors as a good marker of tailings movement along the Paraopeba River since it was also observed in river sections that were not impacted by the collapse. The high RQs observed for this element might be not only the consequence of the collapse, but also of rains and tailings displacement for victims' rescue.

Since all data for Ag were censored, the RQs were calculated for this metal considering the limits of quantification (LOQ) as the MEC values on Equation 3 (1, 2.5 and 5 µg L^{-1}) and resulted in maximum risks of 9.78 and 263.16, respectively for acute and chronic effects (Table 11). If the concentration of Ag had reached the legal limit defined by the Brazilian and Minas Gerais state legislation (10 µg L^{-1}), maximum risks would be higher than those calculated considering the LOQ. Once Ag was identified by Vergilio *et al*. (2020) as an indicator of B1 dam tailings presence into the Paraopeba River, it is critical to implement more sensitive detection and quantification analytical methods, in order to identify and quantify smaller concentrations of this metal, closer to the PNEC values observed in the present study (Table 8).

Highest RQs observed for As, Cd, Cr, Hg and Zn, occurred, respectively in PT-43 (08/09/2020), PT-15 (11/11/2019), PT-28 (19/09/2019), PT-13 (03/07/2020) and in PT-54 (02/12/2019). These values are probably not linked to the B1 dam collapse, since they occurred in monitoring stations located quite far from the collapse point or much after the day when the dam collapsed. Although Cd, Cr and Zn are listed as tailings indicators (VERGILIO *et al.*, 2020) there are also other sources that might contribute to the enrichment of these elements in such locations. The Paraopeba River has a history of anthropic contamination and these maximum values may be linked to the resuspension of metals from sediments, to contributions from tributaries or to other activities in practice in the watershed, such as steel industry and mining in other cities (Congonhas, Ouro Preto and Brumadinho), or to automobile, food and petrochemical industries in the Metropolitan Area of Belo Horizonte (SOARES; PINTO; OLIVEIRA, 2020; THOMPSON *et al.*, 2020).

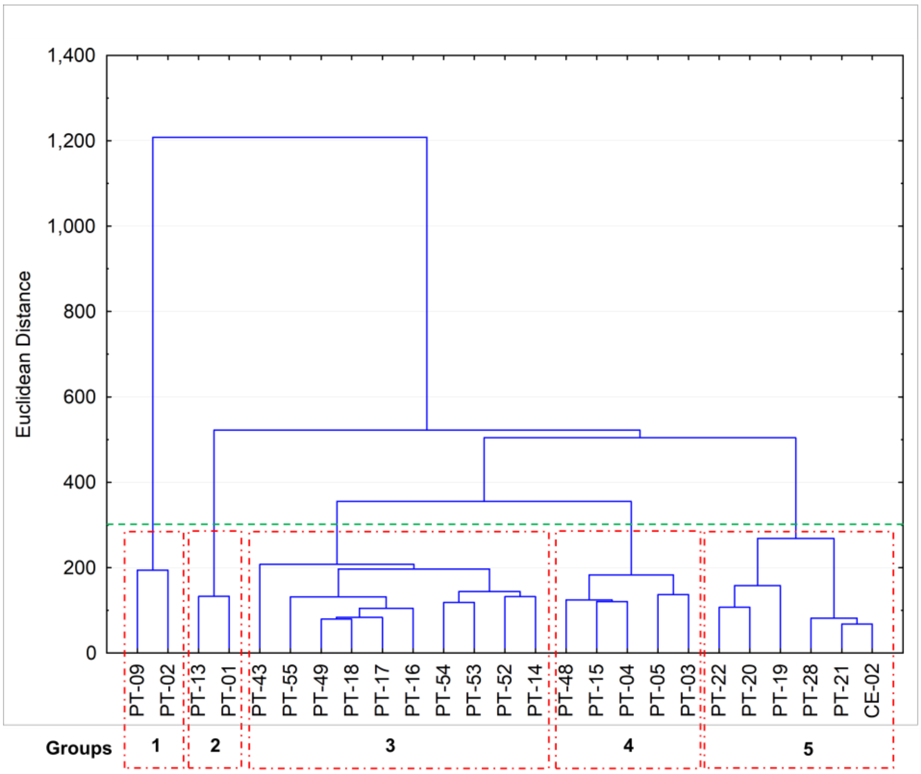
In PT-05, located about 17.5 km downstream the collapse point, maximum RQs were observed for Mn (20.99 and 209.94 for acute and chronic, respectively) and U (0.31 and 3.14, respectively for acute and chronic effects), both in 31/01/2019. Soares *et al*. (2020) found 46.27 mg L^{-1} as the maximum concentration of Mn in the Paraopeba River after the dam collapse (from January 2019 to March 2020) in a monitoring station located 24.8 km downstream the B1 tailings dam. Considering the calculated PNEC values for acute and chronic effects of Mn observed in the present study (366.77 and 36.68 µg L^{-1}, respectively), resultant RQs (126.16 - acute and 1,261.5 – chronic) would be even higher than those observed in this study. As shown in Table 5, Mn is one of the elements which presented highest violation of legal limits in the database used in this study and was pointed out as an indicator of tailings from B1 dam. Hence, high RQs detected in PT-05 for this element might be a consequence of the rupture.

5.4 Assessment of acute and chronic risks in the Paraopeba River water after the B1 dam collapse

5.4.1 Paraopeba River regions

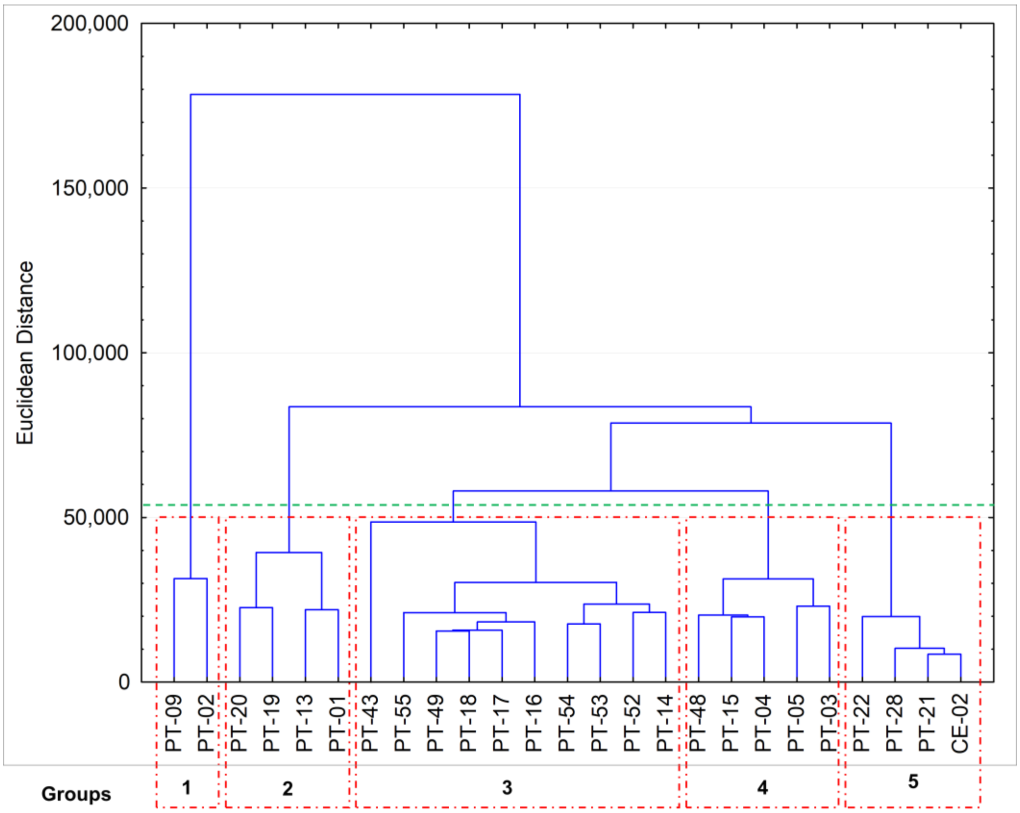
Since acute and chronic RQs for Al, Fe, Mn and Zn presented less than 80% of censored data, Cluster Analysis (CA) was performed for these metals resulting in the acute and chronic dendrograms presented in Figures 10 and 11. The cutting line was defined as 300 for acute risk and 52,000 for chronic risks, resulting in 5 groups.

Figure 10 – Cluster Analysis dendrogram for acute risks associated to the occurrence of Al, Fe, Mn and Zn in the 25 monitoring points between 2019 and 2022



Source: author (2023)

Figure 11 – Cluster Analysis dendrogram for chronic risks associated to the occurrence of Al, Fe, Mn and Zn in the 25 monitoring points between 2019 and 2022



Source: author (2023)

Differences between acute and chronic dendrograms were observed for Group 2. This group was formed by PT-13 and PT-01 for acute RQs, while stations PT-19 and PT-20 were in Group 5. Considering chronic risks, all these four stations were grouped in Group 2. PT-19 is located downstream Pardo River and PT-20 is the first point in the Retiro Baixo Hydroelectric reservoir. In order to keep the same pattern used for group comparisons for acute and chronic risks, PT-19 and PT-20 were not considered in the division of Paraopeba River into regions, based on Cluster Analysis.

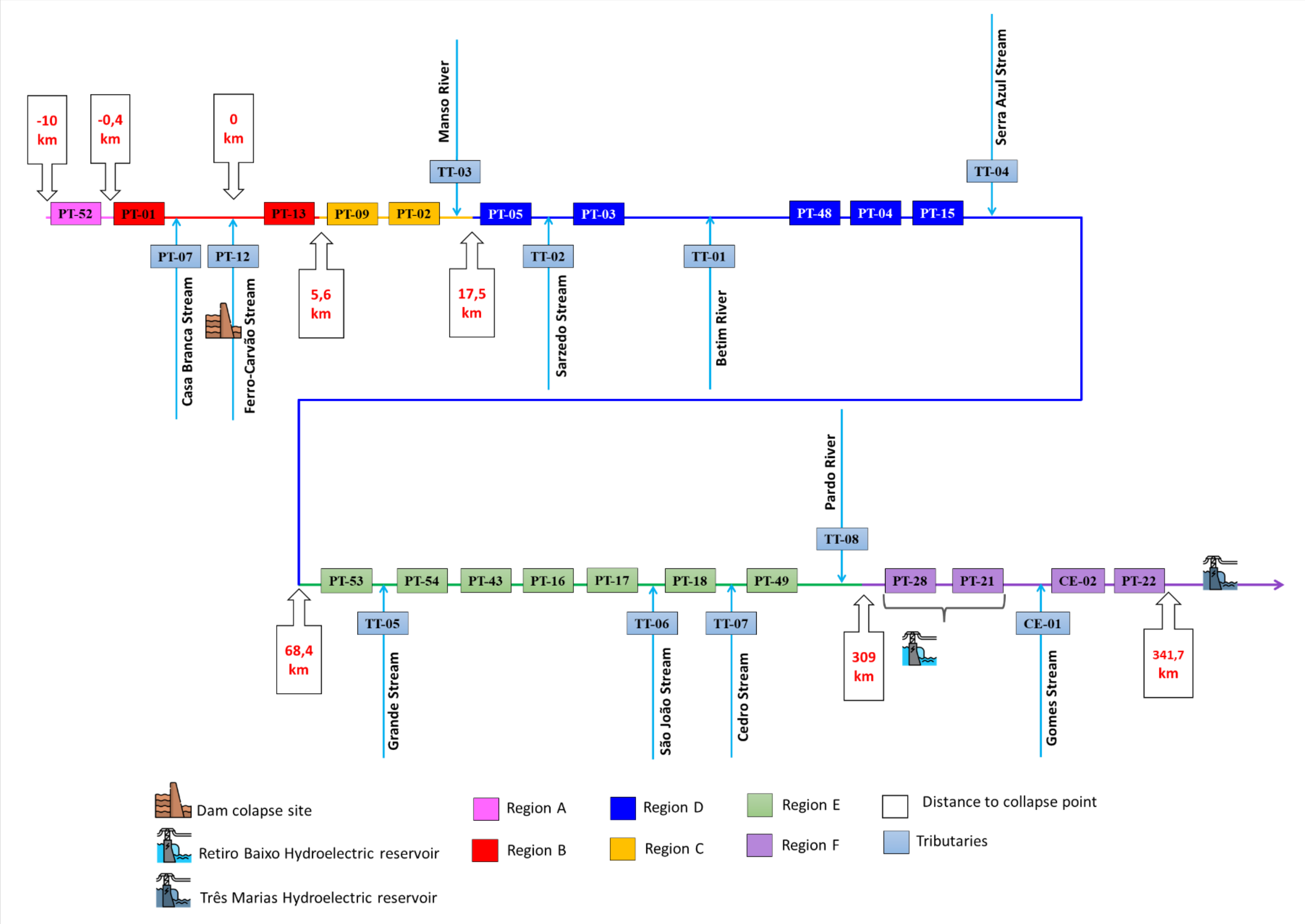
Despite situated geographically closer to stations clustered as Group 4, PT-14 was more similar to stations clustered as Group 3 (Figures 10 and 11). The same occurred with PT-55, which clustered in Group 3, despite located closer to stations clustered as Group 5. PT-14 is located in the confluence of the Betim River, that is highly impacted by anthropic activities (SOARES *et al.*, 2020; SOARES; OLIVEIRA; GOMES, 2022), with the Paraopeba River. This probably influenced the grouping of PT-14 by CA. Similarly, PT-55 is located near the Retiro Baixo backwater area which influences water quality and sediment resuspension and this might have influenced grouping by the CA. In this way, considering the river dynamics and the water flow direction, these two monitoring points were not considered in the Paraopeba River division in regions for spatial and temporal assessment of RQ evolution.

Although PT-52 clustered in Group 3, it is located upstream of the B1 collapse point. Thus, considering the river dynamics and in order to have an upstream comparison region, the PT-52 was included in a different region (RA) than the other stations of Group 3.

PT-01, located about 400 m upstream the confluence between Ferro-Carvão stream and the Paraopeba River might have suffered the influence of the tailings release from the dam. This justifies the fact that this monitoring station was shown to be more similar to PT-13 (200 m downstream the collapse point) compared to other point upstream (PT-52).

Considering results obtained by the CA and the above-mentioned considerations, the Paraopeba River was divided into 6 different regions (RA, RB, RC, RD, RE and RF), as presented in the line diagram (Figure 12), that were used to evaluate the temporal and spatial evolution of the acute and chronic risks.

Figure 12 – Line diagram of monitoring points in the Paraopeba River divided into seven regions based on the Cluster Analysis results



Source: author (2023)

5.4.2 Evolution of acute and chronic risks associated to metals in different regions of the Paraopeba River

Table 12 presents the groups used for spatial evaluation of ecotoxicological risks associated to metals in the Paraopeba River based on regions defined from the CA (Figure 12) and also considering the dry and wet periods between 2019 and 2022.

Table 12 – Spatial and temporal sections of Paraopeba River according to the regions pointed out by the CA and hydrological periods between 2019 e 2022

Regions*	Period**	Comparison Groups	Period**	Comparison Groups
RA	W19	RA-W19	D19	RA-D19
RB		RB-W19		RB-D19
RC		RC-W19		RC-D19
RD		RD-W19		RD-D19
RE		RE-W19		RE-D19
RF		RF-W19		RF-D19
RA	W19-20	RA-W19-20	D20	RA-D20
RB		RB-W19-20		RB-D20
RC		RC-W19-20		RC-D20
RD		RD-W19-20		RD-D20
RE		RE-W19-20		RE-D20
RF		RF-W19-20		RF-D20
RA	W20-21	RA-W20-21	D21	RA-D21
RB		RB-W20-21		RB-D21
RC		RC-W20-21		RC-D21
RD		RD-W20-21		RD-D21
RE		RE-W20-21		RE-D21
RF		RF-W20-21		RF-D21
RA	W21-22	RA-W21-22		
RB		RB-W21-22		
RC		RC- W21-22		
RD		RD- W21-22		
RE		RE- W21-22		
RF		RF- W21-22		

*Regions: RA = Region A; RB = Region B; RC = Region C; RD = Region D; RE = Region E; RF = Region F; **Periods: W19= wet period 2019; D19 = dry period 2019; W19-20 = wet period 2019-2020; D20 = dry period 2020; W20-21 = wet period 2020-2021; D21 = dry period 2021; W21-22 = wet period 2021-2022;

Source: author (2023)

In order to evaluate significant differences among regions RA, RB, RC, RD, RE and RF, inside each period of hydrological years, the Shapiro-Wilk test was applied and showed that data followed asymmetrical distribution. Thus, indicating the appropriateness of using nonparametric statistical techniques.

Figure 13 shows the spatial evolution of acute and chronic risks related to Al, Fe, Mn and Zn, simultaneously, along the Paraopeba River during the first wet season after the B1 dam collapse (W19). For both acute and chronic results, the risks were not significantly different in regions RB, RC and RD. However, these regions presented higher risks than RA, RE and RF, which are located upstream (RA) and further downstream the former dam region (RE and RF), thus indicating that the B1 dam collapse may have increased the ecotoxicological risks in regions located immediately downstream the dam (RB, RC and RD) in the sequence of the disaster. Besides, as regions RA, RE and RF were not significantly different, it is possible that tailings did not increase RQs in regions RE and RF between January and March 2019 (W19).

During the dry period of 2019 (D19), from April to September, the multiple comparison Dunn's test results for acute and chronic effects showed similar results to those observed in W-19, except for the fact that risks obtained for RF were significantly smaller when compared to RA (Figure 14). Thus, regions RB, RC and RD were still the most impacted 3 months after the B1 dam collapse and tailings had probably not reached RE and RF. Despite similar results between W19 and D19, maximum observed risks for the dry season were smaller than those observed for W19, indicating that part of the tailings probably had settled in the Paraopeba River channel, due to smaller turbulence and volume of water during the dry period.

In contrast, maximum acute and chronic risks for region RD (Figure 15) were smaller in W19-20 (56.35 and 9,263 – acute and chronic, respectively) than in D19 (321.09 and 45,285, acute and chronic, respectively) (Figure 14). As there is high influence of the Betim River in this region and dilution potential is reduced in the dry season, higher risks probably occurred due to the influence of this tributary.

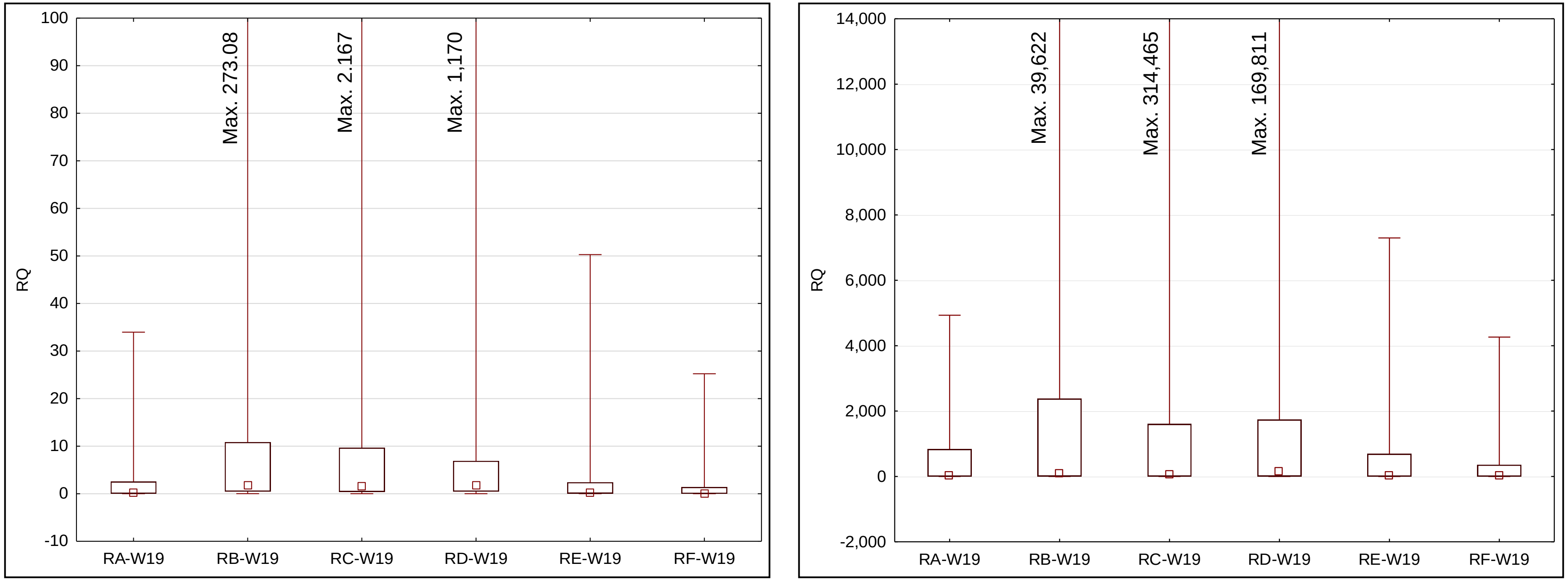
In W19-20, the Dunn's test results considering risks of acute toxicity were similar to those observed for risks of chronic toxicity (Figure 15), except between RB and RC; between RA and RF; and between RE and RF. Likewise for W19 and D19 (Figures 13 and 14), ecotoxicological risks observed for regions RB, RC and RD in W19-20 (Figure 15) were higher than in RA, showing that these regions were still impacted by the B1 dam collapse. In W19-20, risks observed for region RD were significantly smaller than regions RB and RC, thus showing a different pattern when compared to previous periods W19 and D19, when risks observed in RD were similar to those observed in RB and RC. Despite that, RQs in regions RE and RF were still significantly smaller than the RQs in regions RB, RC and RD for W19-20 when compared to the two first seasons after the collapse (Figures 13 and 14).

Similarly to results observed in W19-20, the multiple comparison Dunn's test showed similar results considering acute and chronic risks observed in the dry period of 2020 (D20) (Figure 16). The main difference between W19-20 and D20 was that during the wet period, RE was significantly smaller than RD, while during the dry season, they were not significantly different. Similar to the previous periods, RQs in regions RB, RC and RD were significantly higher than the risks in regions RA, RE and RF, thus suggesting that main impacts of the tailings dam collapse remained in these regions during D20. Nevertheless, maximum risks in D20 were smaller than during all previous evaluated periods (W19, D19, W19-20), thus indicating water quality depuration throughout time (Figures 13, 14 and 15).

Regarding the wet season between 2020 and 2021 (W20-21) (Figure 17), there were significant differences for acute risks between regions RB, RC and RF when compared to region RA, between RE and RF when compared to regions RB and RC, and between region RF when compared to regions RD and RE. However, based on median values, it was not possible to affirm which region presented the highest RQs. For chronic risks during W20-21 (Figure 17), the main difference between the previous evaluated season (D20 - Figure 16) was in region RD which did not differ significantly to RA and RB in W20-21, thus reinforcing depuration tendencies.

For the dry period of 2021 (D21) and wet period of 2021-2022 (W21-22), (Figures 18 and 19, respectively), RF was the only region for which ecotoxicological risks were significantly different from others. For D21 acute and chronic effects and W21-22 acute effects, it was not possible, based on RQs median values, to affirm which region presented the highest RQs. For chronic effects in W21-22, RF was significantly smaller than RA, RD and RE and higher than RB and RC. The observed results for these two last periods, indicated that tailings might have caused alteration in the RQs in the region of Retiro Baixo hydroelectric reservoir (RF) by D21 and the risks along the Paraopeba River decreased. However, it is not possible to confirm such assumption without the evaluation of a longer database containing data obtained previously to the collapse.

Figure 13 – Spatial evolution of acute (left) and chronic (right) risks along the Paraopeba River during the wet period of 2019 (W19)



	RA-W19	RB-W19	RC-W19	RD-W19	RE-W19	RF-W19
RA-W19						
RB-W19	↑					
RC-W19	↑	=				
RD-W19	↑	=	=			
RE-W19	=	↓	↓	↓		
RF-W19	=	↓	↓	↓	↓	

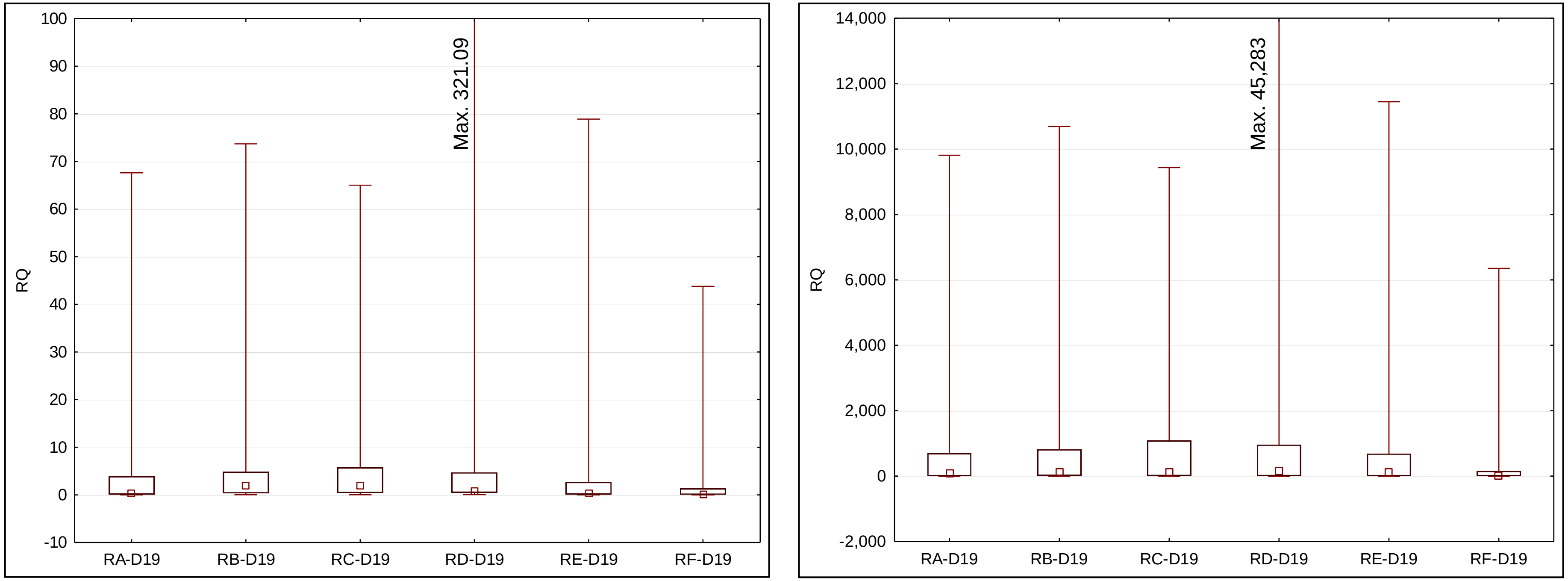
	RA-W19	RB-W19	RC-W19	RD-W19	RE-W19	RF-W19
RA-W19						
RB-W19	↑					
RC-W19	↑	=				
RD-W19	↑	=	=			
RE-W19	=	↓	↓	↓		
RF-W19	=	↓	↓	↓	*	

= Underlined group (at left) does not differ significantly from the group in bold (above)
↑ Underlined group (at left) is significantly higher than the group in bold (above)
↓ Underlined group (at left) is significantly higher than the group in bold (above)
* Underlined group (at left) is significantly different than the group in bold (above), but based on median values, it was not possible to identify the highest.

Median
25%-75%
Min-Max

Source: author (2023).

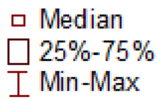
Figure 14 – Spatial evolution of acute (left) and chronic (right) risks along the Paraopeba River during the dry period of 2019 (D19)



	RA-D19	**RB-D19**	**RC-D19**	**RD-D19**	**RE-D19**	**RF-D19**
RA-D19						
RB-D19	↑					
RC-D19	↑	=				
RD-D19	↑	=	=			
RE-D19	=	↓	↓	↓		
RF-D19	↓	↓	↓	↓	↓	

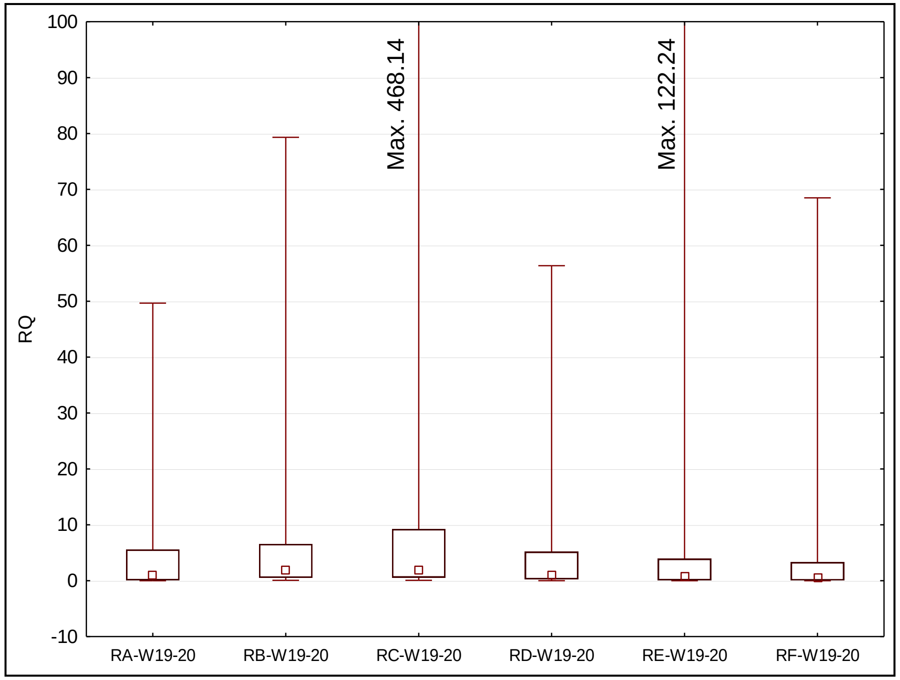
	RA-D19	**RB-D19**	**RC-D19**	**RD-D19**	**RE-D19**	**RF-D19**
RA-D19						
RB-D19	↑					
RC-D19	↑	=				
RD-D19	↑	=	=			
RE-D19	=	↓	↓	↓		
RF-D19	↓	↓	↓	↓	↓	

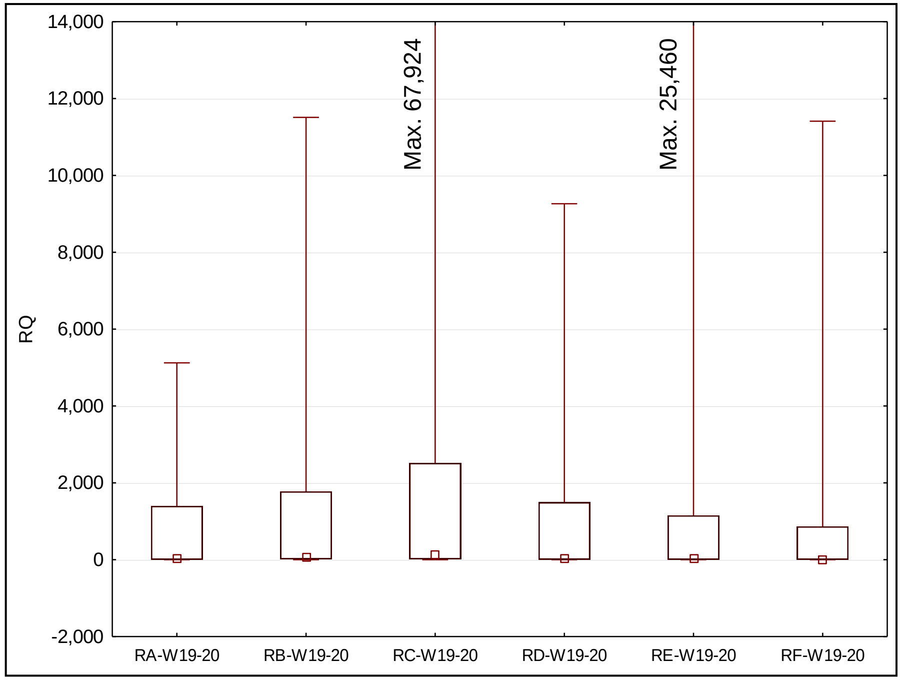
= Underlined group (at left) does not differ significantly from the group in bold (above)
↑ Underlined group (at left) is significantly higher than the group in bold (above)
↓ Underlined group (at left) is significantly higher than the group in bold (above)
* Underlined group (at left) is significantly different than the group in bold (above), but based on median values, it was not possible to identify the highest.



Source: author (2023)

Figure 15 – Spatial evolution of acute (left) and chronic (right) risks along the Paraopeba River during the wet period of 2019-2020 (W19-20)





	RA-W19-20	**RB-W19-20**	**RC-W19-20**	**RD-W19-20**	**RE-W19-20**	**RF-W19-20**
RA-W19-20						
RB-W19-20	↑					
RC-W19-20	↑	=				
RD-W19-20	↑	↓	↓			
RE-W19-20	=	↓	↓	↓		
RF-W19-20	↓	↓	↓	↓	↓	

	RA-W19-20	**RB-W19-20**	**RC-W19-20**	**RD-W19-20**	**RE-W19-20**	**RF-W19-20**
RA-W19-20						
RB-W19-20	↑					
RC-W19-20	↑	↑				
RD-W19-20	↑	↓	↓			
RE-W19-20	=	↓	↓	↓		
RF-W19-20	=	↓	↓	↓	=	

= Underlined group (at left) does not differ significantly from the group in bold (above)
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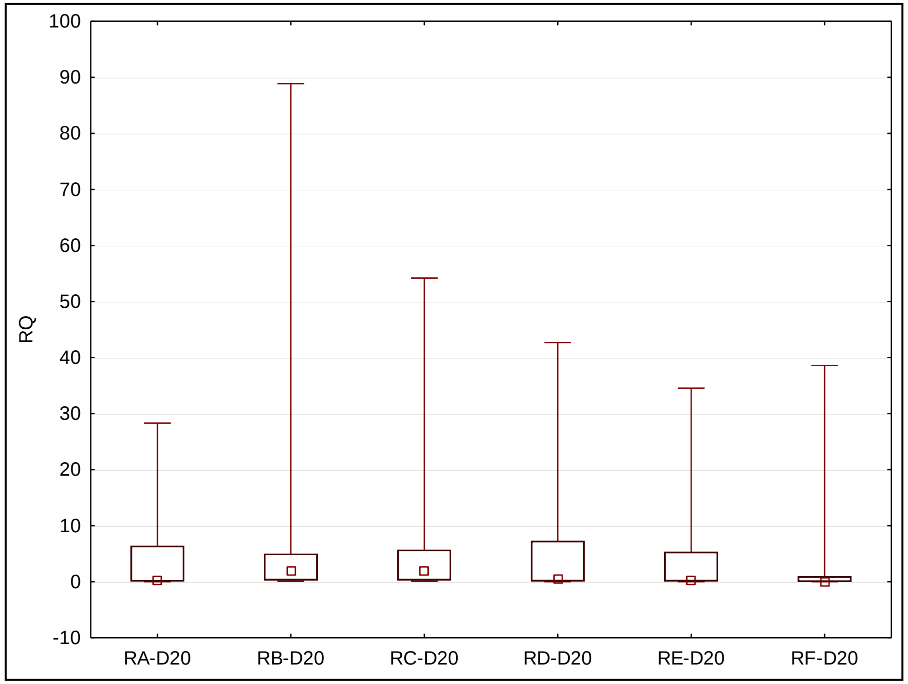
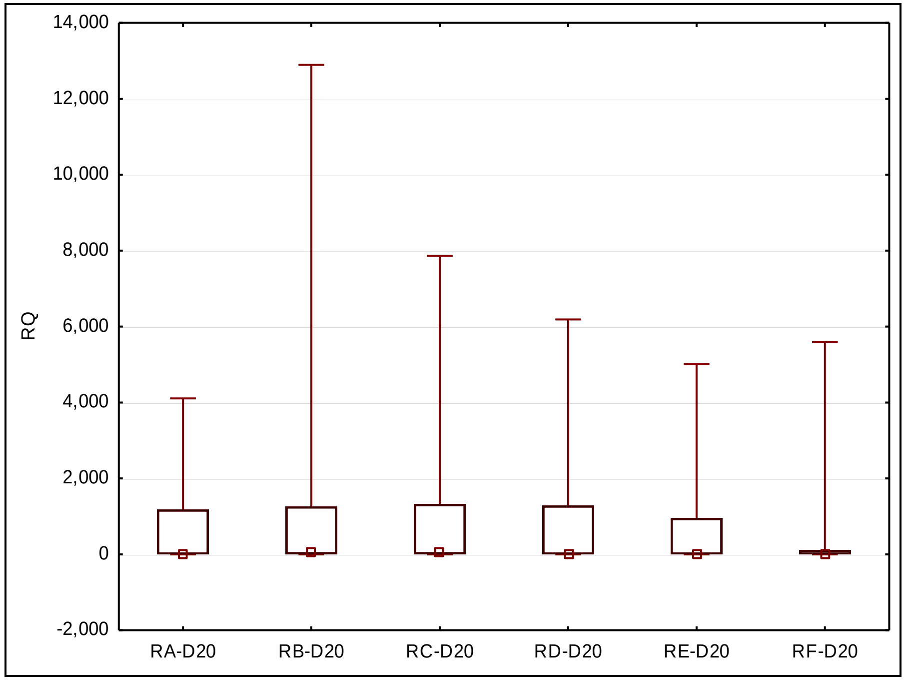
↓ Underlined group (at left) is significantly higher than the group in bold (above)
* Underlined group (at left) is significantly different than the group in bold (above), but based on median values, it was not possible to identify the highest.

Median
25%-75%
Min-Max

Source: author (2023)

Figure 16 – Spatial evolution of acute (left) and chronic (right) risks along the Paraopeba River during the dry period of 2020 (D20)





	RA-D20	**RB-D20**	**RC-D20**	**RD-D20**	**RE-D20**	**RF-D20**
RA-D20						
RB-D20	↑					
RC-D20	↑	=				
RD-D20	↑	↓	↓			
RE-D20	=	↓	↓	=		
RF-D20	↓	↓	↓	↓	↓	

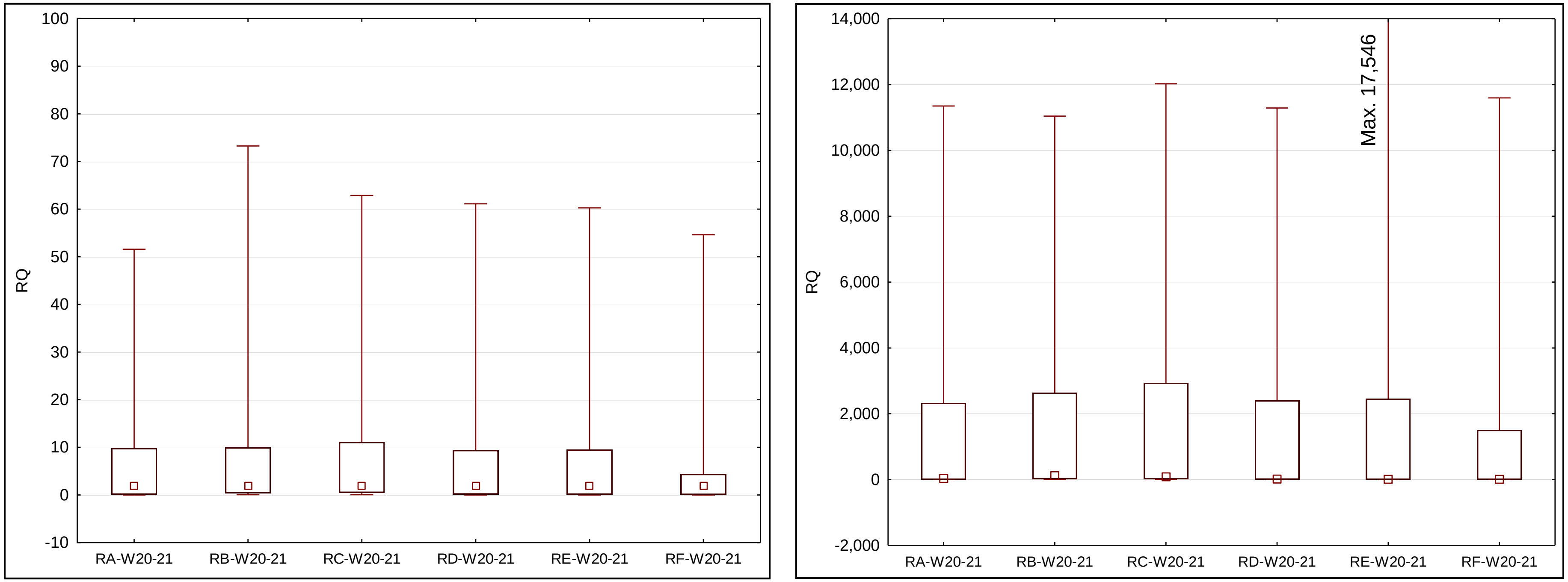
	RA-D20	**RB-D20**	**RC-D20**	**RD-D20**	**RE-D20**	**RF-D20**
RA-D20						
RB-D20	↑					
RC-D20	↑	=				
RD-D20	↑	↓	↓			
RE-D20	=	↓	↓	=		
RF-D20	↓	↓	↓	↓	↓	

= Underlined group (at left) does not differ significantly from the group in bold (above)
↑ Underlined group (at left) is significantly higher than the group in bold (above)
↓ Underlined group (at left) is significantly higher than the group in bold (above)
* Underlined group (at left) is significantly different than the group in bold (above), but based on median values, it was not possible to identify the highest.

□ Median
□ 25%-75%
⌶ Min-Max

Source: author (2023)

Figure 17 – Spatial evolution of acute (left) and chronic (right) risks along the Paraopeba River during the wet period of 2020-2021 (W20-21)



	RA-W20-21	**RB-W20-21**	**RC-W20-21**	**RD-W20-21**	**RE-W20-21**	**RF-W20-21**
RA-W20-21						
RB-W20-21	*					
RC-W20-21	*	=				
RD-W20-21	=	=	=			
RE-W20-21	=	*	*	=		
RF-W20-21	*	*	*	*	*	

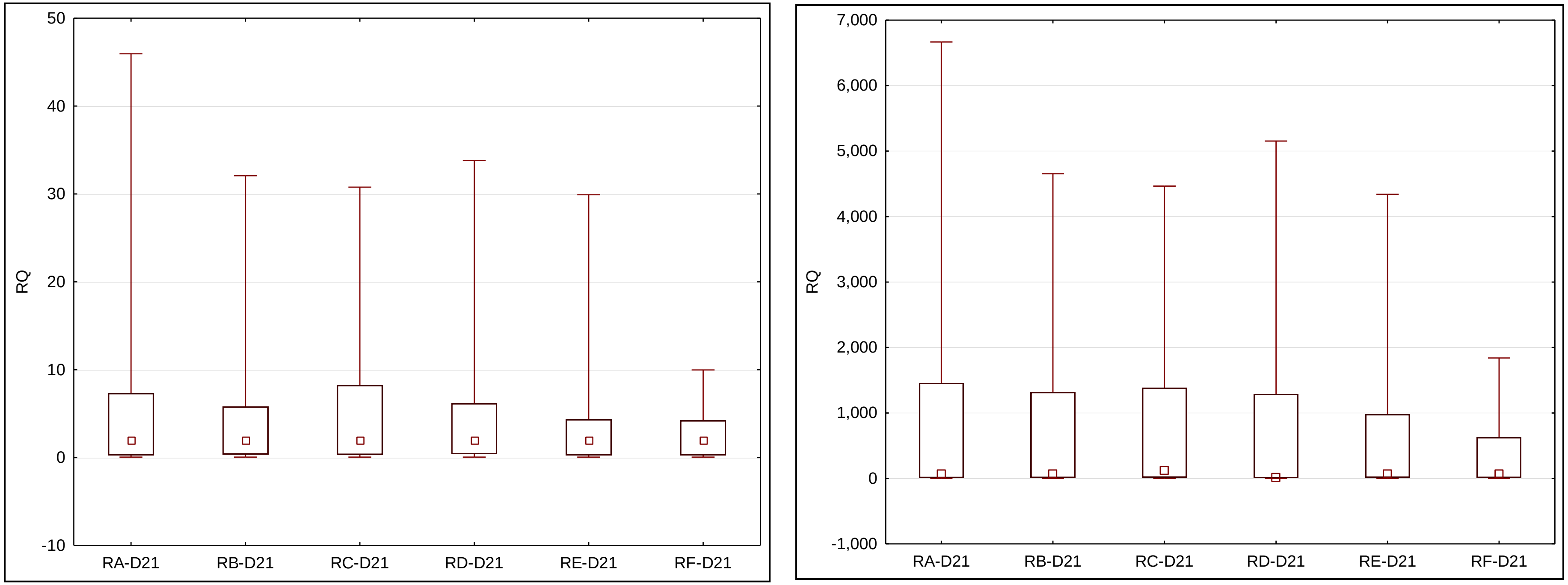
	RA-W20-21	**RB-W20-21**	**RC-W20-21**	**RD-W20-21**	**RE-W20-21**	**RF-W20-21**
RA-W20-21						
RB-W20-21	↑					
RC-W20-21	↑	=				
RD-W20-21	=	=	↓			
RE-W20-21	=	↓	↓	=		
RF-W20-21	↓	↓	↓	↓	*	

= Underlined group (at left) does not differ significantly from the group in bold (above)
↑ Underlined group (at left) is significantly higher than the group in bold (above)
↓ Underlined group (at left) is significantly higher than the group in bold (above)
* Underlined group (at left) is significantly different than the group in bold (above), but based on median values, it was not possible to identify the highest.

Median
25%-75%
Min-Max

Source: author (2023)

Figure 18 – Spatial evolution of acute (left) and chronic (right) risks along the Paraopeba River during the dry period of 2021 (D21)



	RA-D21	**RB-D21**	**RC-D21**	**RD-D21**	**RE-D21**	**RF-D21**
RA-D21						
RB-D21	=					
RC-D21	=	=				
RD-D21	=	=	=			
RE-D21	=	=	=	=		
RF-D21	*	*	*	*	*	

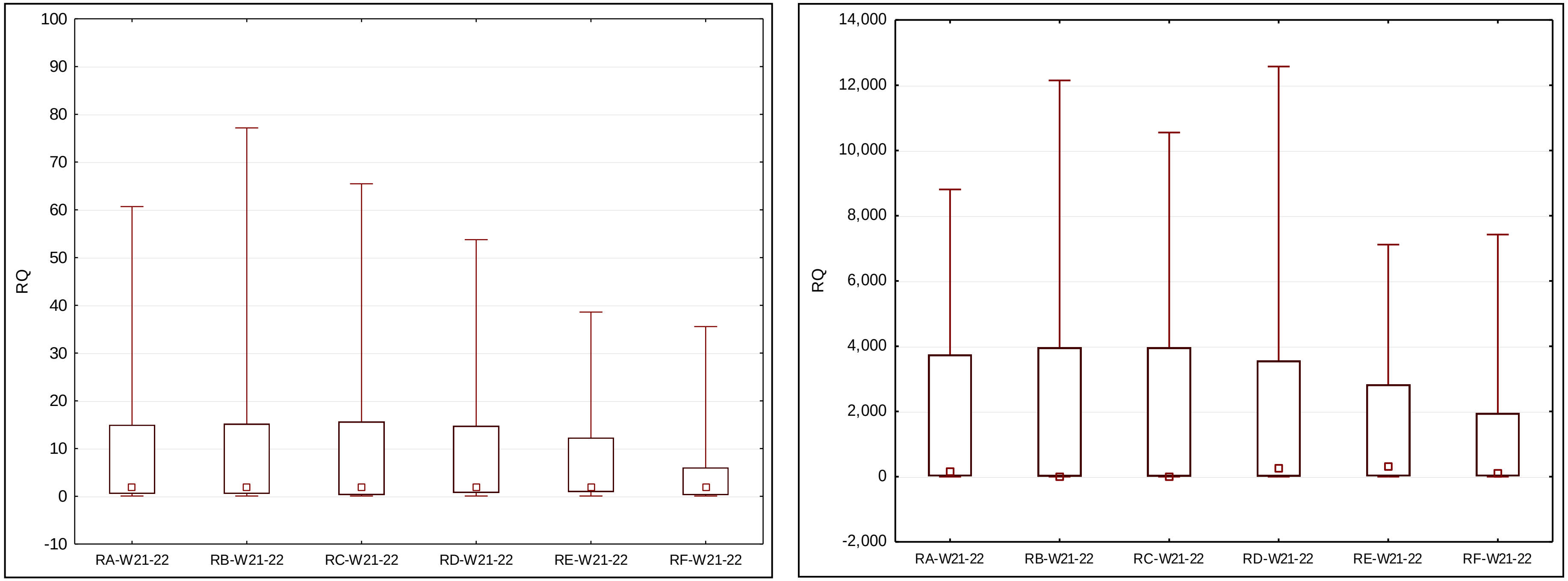
	RA-D21	**RB-D21**	**RC-D21**	**RD-D21**	**RE-D21**	**RF-D21**
RA-D21						
RB-D21	=					
RC-D21	=	=				
RD-D21	=	=	=			
RE-D21	=	=	=	=		
RF-D21	*	*	*	*	*	

= Underlined group (at left) does not differ significantly from the group in bold (above)
↑ Underlined group (at left) is significantly higher than the group in bold (above)
↓ Underlined group (at left) is significantly higher than the group in bold (above)
* Underlined group (at left) is significantly different than the group in bold (above), but based on median values, it was not possible to identify the highest.

□ Median
□ 25%-75%
⌶ Min-Max

Source: author (2023)

Figure 19 – Spatial evolution of acute (left) and chronic (right) risks along the Paraopeba River during the wet period of 2021-2022 (W21-22)



	RA-W21-22	**RB-W21-22**	**RC-W21-22**	**RD-W21-22**	**RE-W21-22**	**RF-W21-22**
RA-W21-22						
RB-W21-22	=					
RC-W21-22	=	=				
RD-W21-22	=	=	=			
RE-W21-22	=	=	=	=		
RF-W21-22	*	*	*	*	=	

	RA-W21-22	**RB-W21-22**	**RC-W21-22**	**RD-W21-22**	**RE-W21-22**	**RF-W21-22**
RA-W21-22						
RB-W21-22	=					
RC-W21-22	=	=				
RD-W21-22	=	=	=			
RE-W21-22	=	=	=	=		
RF-W21-22	↓	↑	↑	↓	↓	

= Underlined group (at left) does not differ significantly from the group in bold (above)
↑ Underlined group (at left) is significantly higher than the group in bold (above)
↓ Underlined group (at left) is significantly higher than the group in bold (above)
* Underlined group (at left) is significantly different than the group in bold (above), but based on median values, it was not possible to identify the highest.

□ Median
□ 25%-75%
Min-Max

Source: author (2023)

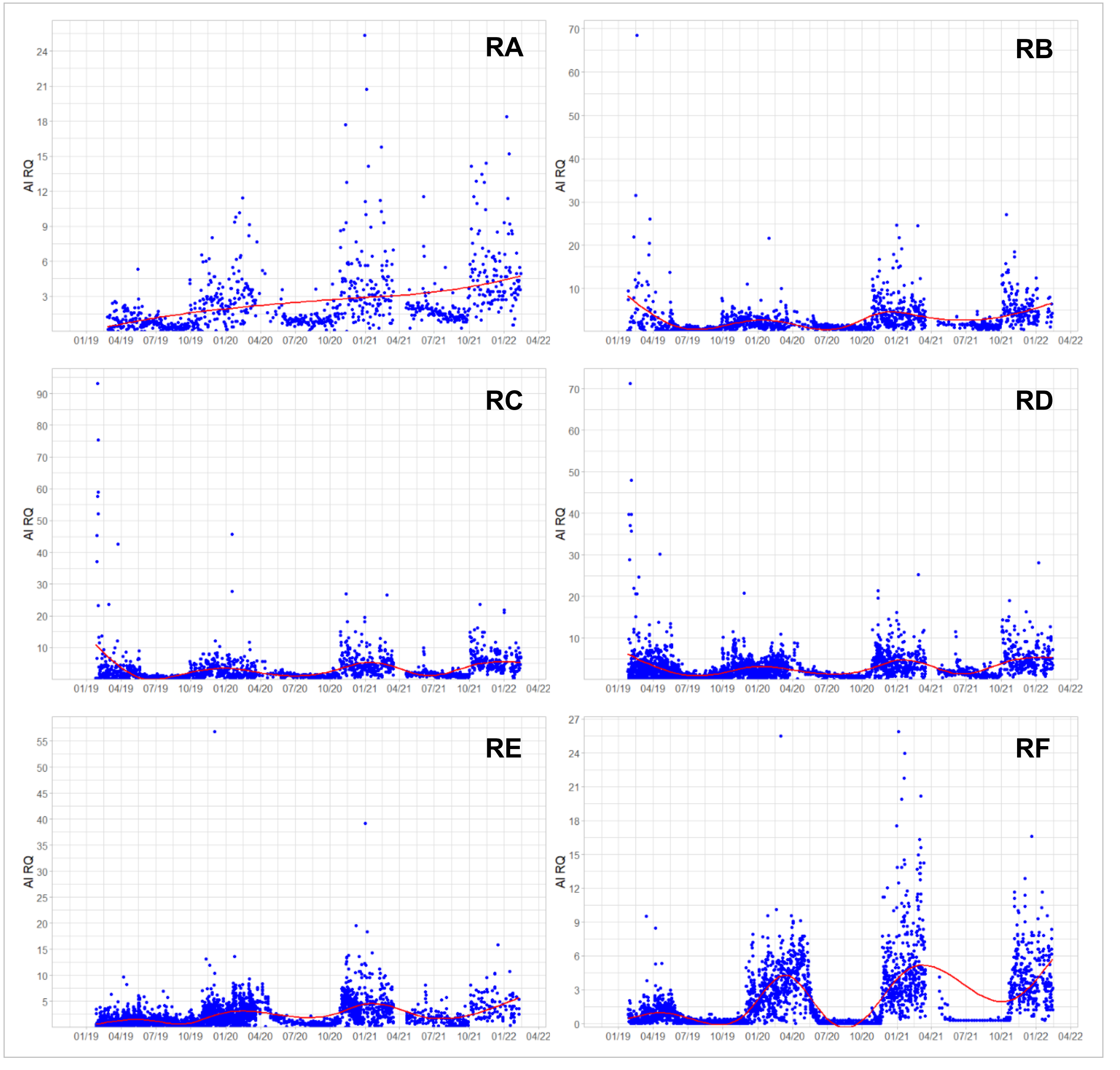
5.4.3 Temporal profiles of acute and chronic ecotoxicological risks of metals in the Paraopeba River water

Figures 20 to 27 present the time series for acute and chronic RQs related to Al, Fe, Mn and Zn in the Paraopeba River according to the regions A to F (Figure 12) after the B1 dam collapse. It is important to observe that vertical axis have different scales in each graph.

For most of the time series, it is clear that RQs follow a seasonal pattern with peaks during the wet season (October to March) and valleys in the dry season (April to September) (FERREIRA *et al.*, 2021). These peaks and valleys can be generally related to the modification of the water flow and volume in the river channel, which is higher during the wet season, increasing the water turbulence and, consequently, resuspending settled substances from the sediment to the water column. Furthermore, during the wet season, the surface drainage to the river channel is higher than in the dry season, and stormwater may also be an extra source of pollutants, such as metals, to the river (SOARES; OLIVEIRA; GOMES, 2022).

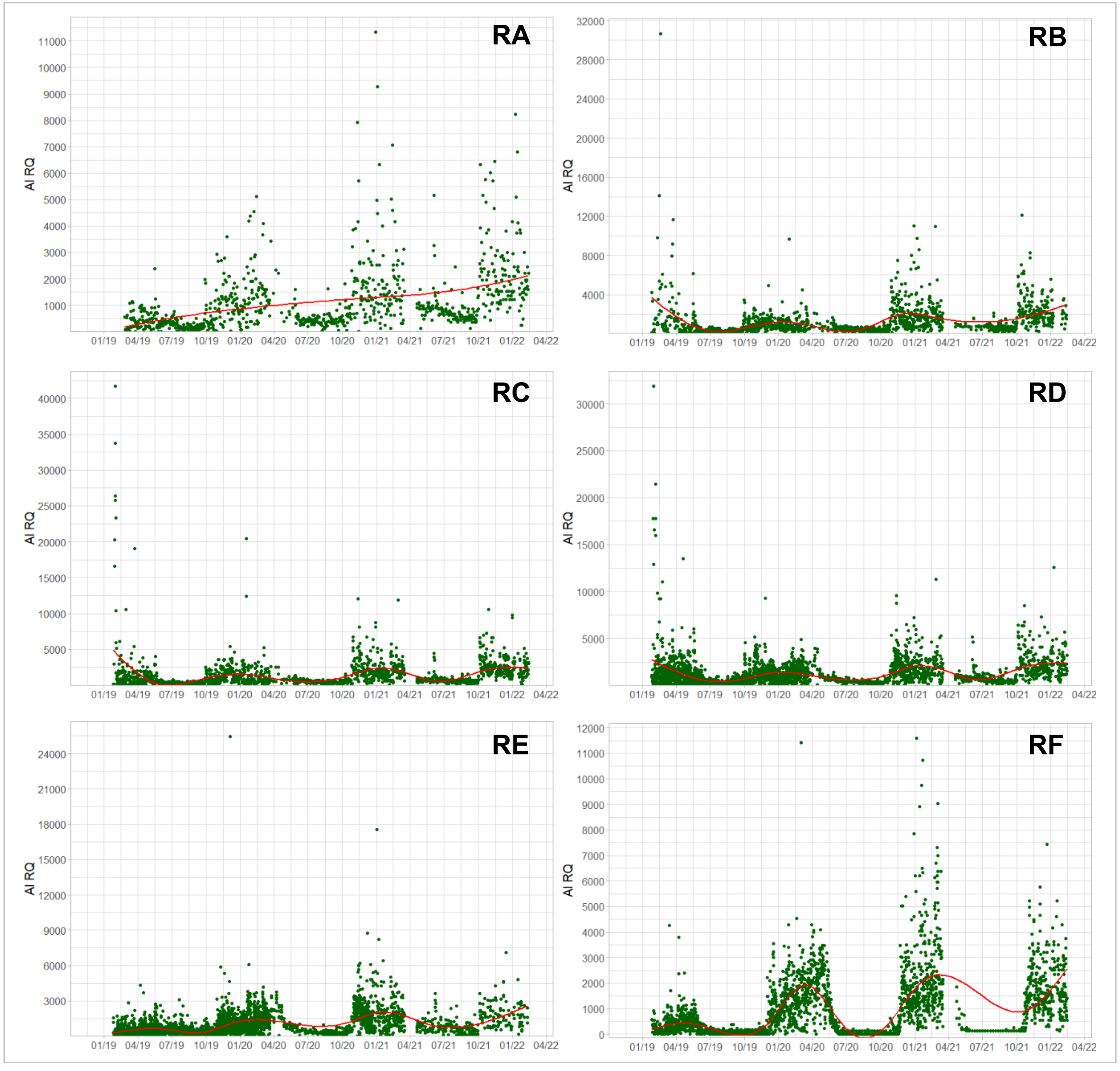
As shown in Figures 20 and 21, the cyclic seasonal variation of RQs for Al are well marked. For region RA, located at the B1 dam upstream area, risks reached maximum values of 25.35 and 11,349 for acute and chronic effects, respectively. Time series RQ profile and maximum values obtained for Al (25.9 – acute and 11,595 – chronic) in region RF are similar to those observed in RA. For regions RB, RC and RD, highest RQs were observed during the three first months after the dam collapse, reducing in the subsequent months, although extreme RQ values were observed for Region C, reaching acute and chronic RQs of, respectively, 45.78 and 20,490, during the wet season of 2019-2020 (W19-20). These observations reflect the proximity of these regions to the B1 dam area. Peak values observed for RQs related to Al in region RE occurred during this same wet season, followed by the peak in the wet period of 2020-2021 (W20-21), probably as a result of tailings transportation along the Paraopeba River.

Figure 20 – Time series of Al acute RQs in Paraopeba River regions A to F



Source: author (2023)

Figure 21 – Time series of Al chronic RQs in Paraopeba River regions A to F



Source: author (2023)

The highest RQs in the Paraopeba River were observed for Fe (Figures 22 and 23), as expected, since this was the mineral extracted in the mining complex and the metal which showed highest concentration in the composition of B1 dam tailings (THOMPSON *et al.*, 2020). Furthermore, the geological formation in the Quadrilátero Ferrífero (Iron Quadrangle region), where study area is located, is intrinsically rich in Fe and, consequently, there is a predominance of mining activities which explore this mineral. Thus, Fe can leach to water bodies, altering the surface water quality and increasing RQs, as reflected in this study by highest RQ values for Fe in region RA, located about 10 km upstream the collapse point, when compared RQs values obtained for the other evaluated metals in the same region (PORSANI; DE JESUS; STANGARI, 2019; SOARES; OLIVEIRA; GOMES, 2022).

Despite the external and natural contributions of Fe to the Paraopeba River, the impacts of the B1 tailings dam collapse in ecotoxicological risks associated to this element is clear in the time series graphs (Figures 22 and 23). Regions RC and RD showed peaks of high risks associated to Fe in dates close to the collapse date and along the wet season of 2019 (W19), reaching maximum values of 2,167 and 314,465 in RC, and of 1,170 and 169,811 in RD, for acute and chronic risks, respectively. For RC, peaks of acute and chronic risks were also observed during the wet period of 2019-2020 (W19-20) reaching maximum values of acute and chronic RQ equivalent to 468.14 and 67,924, respectively. These were smaller than the peaks observed during the first wet season after the dam collapse, thus confirming the impact of the disaster to increased ecotoxicological risks in the region. After initial peaks of RQs associated to Fe in regions RC and RD (Figures 22 and 23) during W19, the profile became similar to that observed in the upstream region (RA), reaching maximum risks of nearly 40 for acute effects and 6,000 for chronic effects.

Peaks of acute and chronic RQs for Fe were also observed in region RB during W19, reaching maximum values of 273.08 and 39,622 for acute and chronic effects, respectively (Figures 22 and 23). Although a decrease in acute and chronic maximum RQs values to about 70 and 10,000, respectively, observed during the wet periods of 2019-2020, 2020-2021 and 2021-2022, these values were higher than peaks verified for the upstream region RA. This might indicate that the region was still under influence of the tailings dam collapse until W21-22, the last period evaluated in the present study.

This influence might be a consequence of activities associated to the repair of the collapse area which involve transportation of soil and tailings in the area, thus impacting nearest regions such as RB.

The time series graphs obtained for RQs related to Fe in RE and RF (Figures 22 and 23) show a pattern which is similar to that observed in RA as well as similar maximum values of acute and chronic RQs. This indicates that these regions were not majorly affected by the dam collapse when it comes to ecotoxicological risks.

Compared to Al, Fe and Zn, RQs obtained for Mn were the smallest (Figures 24 and 25), reaching highest values of 20.99 and 209.94 for acute and chronic effects, respectively, in region RD. For region RA, the average RQs associated to Mn were equivalent to 0.05 and 0.5, respectively, for acute and chronic effects. Constant RQs associated to Mn were observed after January 2021 in region RA probably linked to observed values which were under the LOQ of the analytical method used to quantify this element.

Figures 24 and 25 show that the highest RQs values for Mn in RB occurred during W19, W19-20 and W20-21, decreasing after these periods. Maximum RQs observed in this region were higher than those observed in RA for both acute and chronic effects. As observed for Fe, this indicates that the impact of the B1 dam collapse was not limited to the wet season following the disaster, yet lasted for a complete hydrological year.

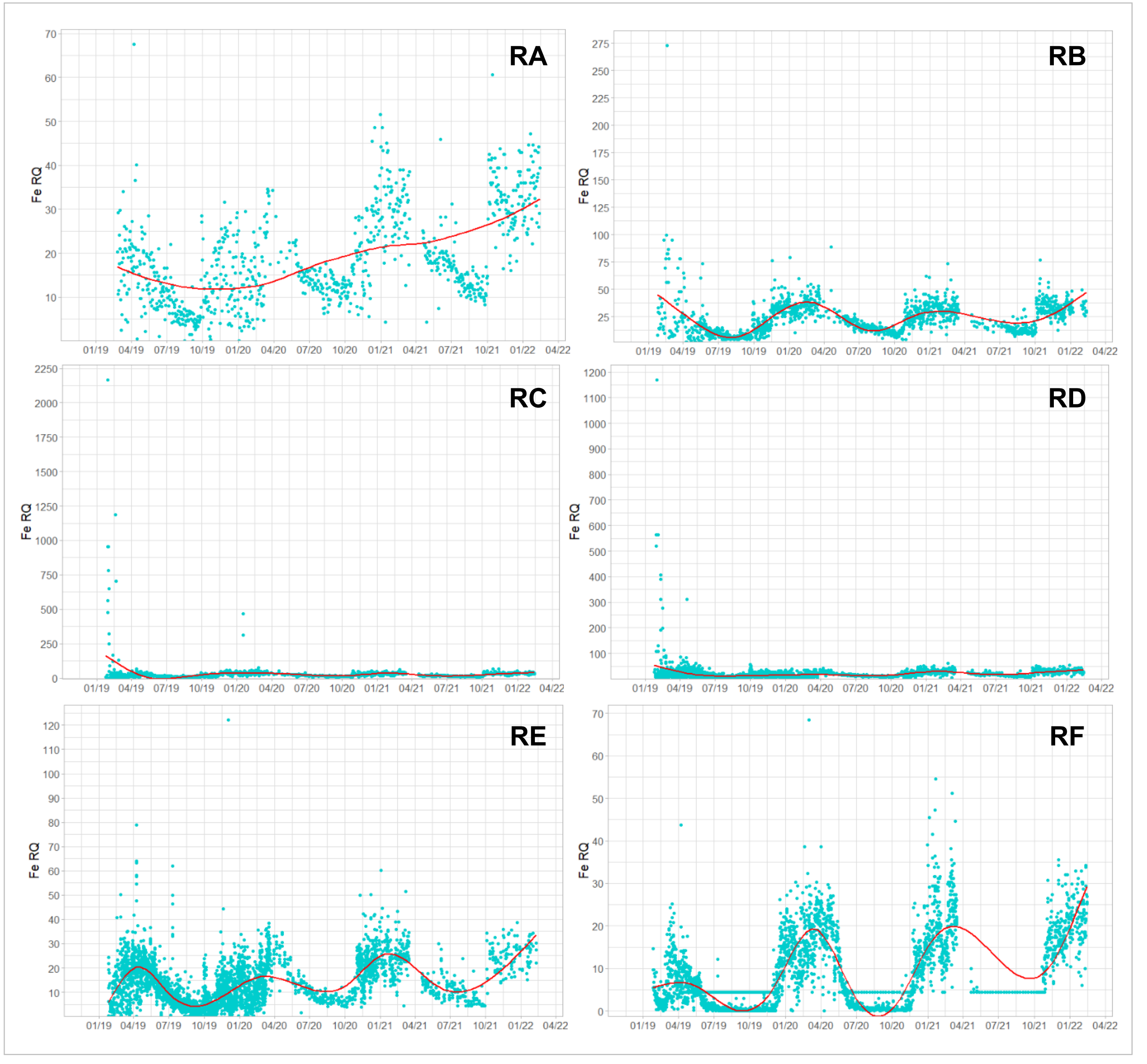
In regions RC and RD (Figures 24 and 25), the highest RQs observed for Mn were detected during the two first wet seasons (W19 and W19-20). After that, RQs decreased and reached values similar to RA. Constant RQs linked to measured concentrations below the LOQ were also observed in RF. However, in contrast to observation made for Al (Figures 20 and 21) risk peaks for Mn in RF were higher than those observed in RA.

Regarding Zn, for both acute and chronic effects, calculated RQs were limited by the LOQ associated to the analytical method used to quantify this metal. This is reflected by constant RQ values observed in Figures 26 and 27. The biggest values occurred during the three first months after the dam collapse for regions RB and RC. Then RQs became constant at 2 for acute and 13 for chronic effect. These values were similar to those observed for RA. For regions RD, RE and RF, peaks were observed from January 2019 to April 2020, when risks reached the same constant values observed in regions RB and RC

The present spatiotemporal analysis of RQs in the Paraopeba River allowed to identify critical regions (RB, RC and RD) related to risks to aquatic biota in the period following B1 dam collapse. Thus, results obtained here might help guiding management actions, in order to prioritize a more frequent ecotoxicological monitoring in these areas, mainly involving the most sensitive organisms and trophic levels identified through SSD curves. It should be noted that the present study can be used as a guide and not as a replacement for laboratory ecotoxicological assays conducted with samples collected in the study area.

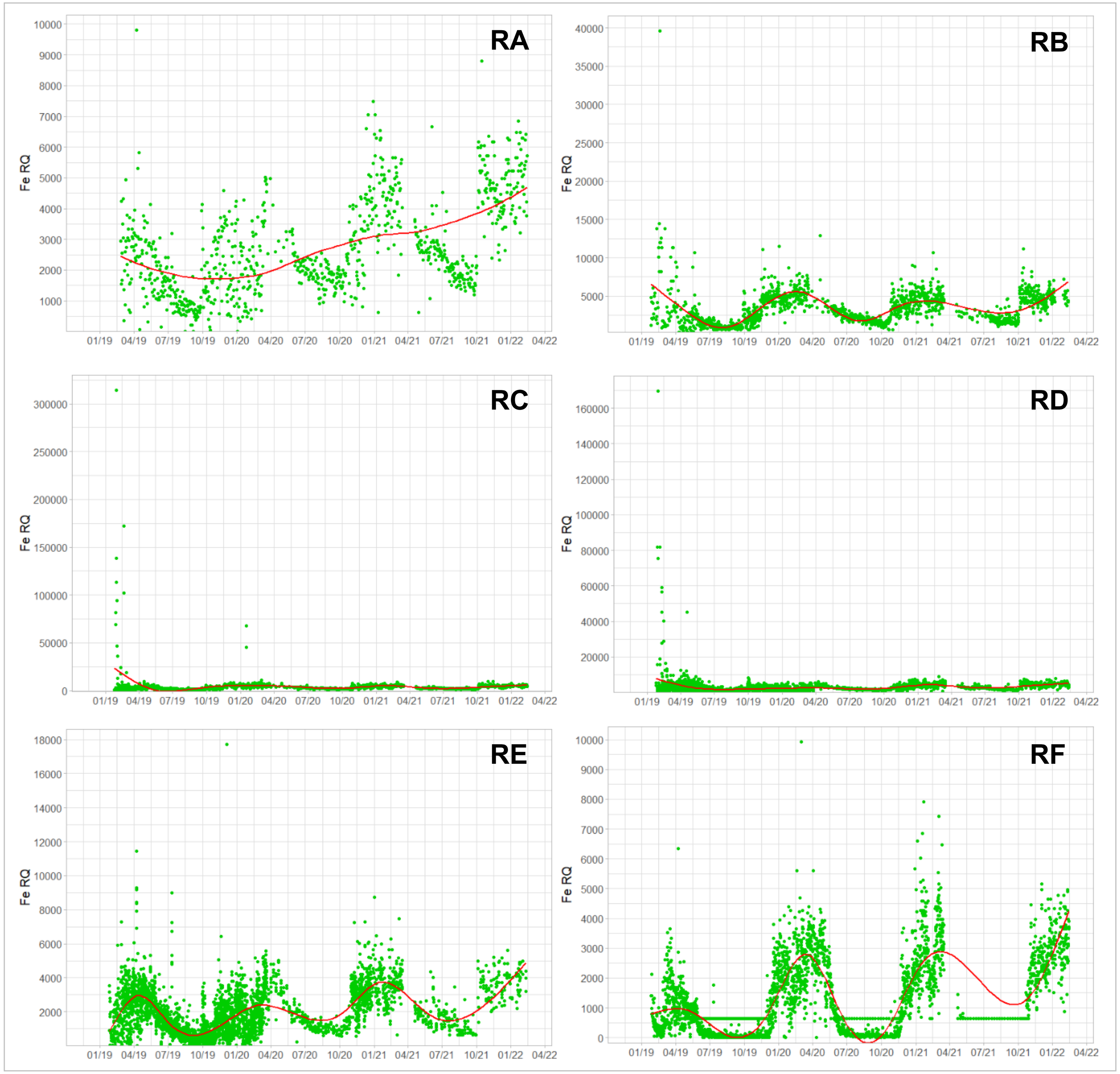
It is important to emphasize that, although results indicated an absence of significant difference in risks throughout time and along the Paraopeba River over the studied period, these risks were not low, so it does not mean that the biodiversity in the river is preserved. During the studied period, high risks were observed even in the upstream region RA due to impacts of anthropic activities in the watershed. Thus, the fact that regions located immediately downstream the B1 dam area reached risks that were not significantly different from RA does not mean that the Paraopeba River has completely recovered from the tailings dam collapse. These risks related to the accident could have been disguised by previous conditions existing in the Paraopeba River.

Figure 22 – Time series of Fe acute RQs in Paraopeba River regions A to F



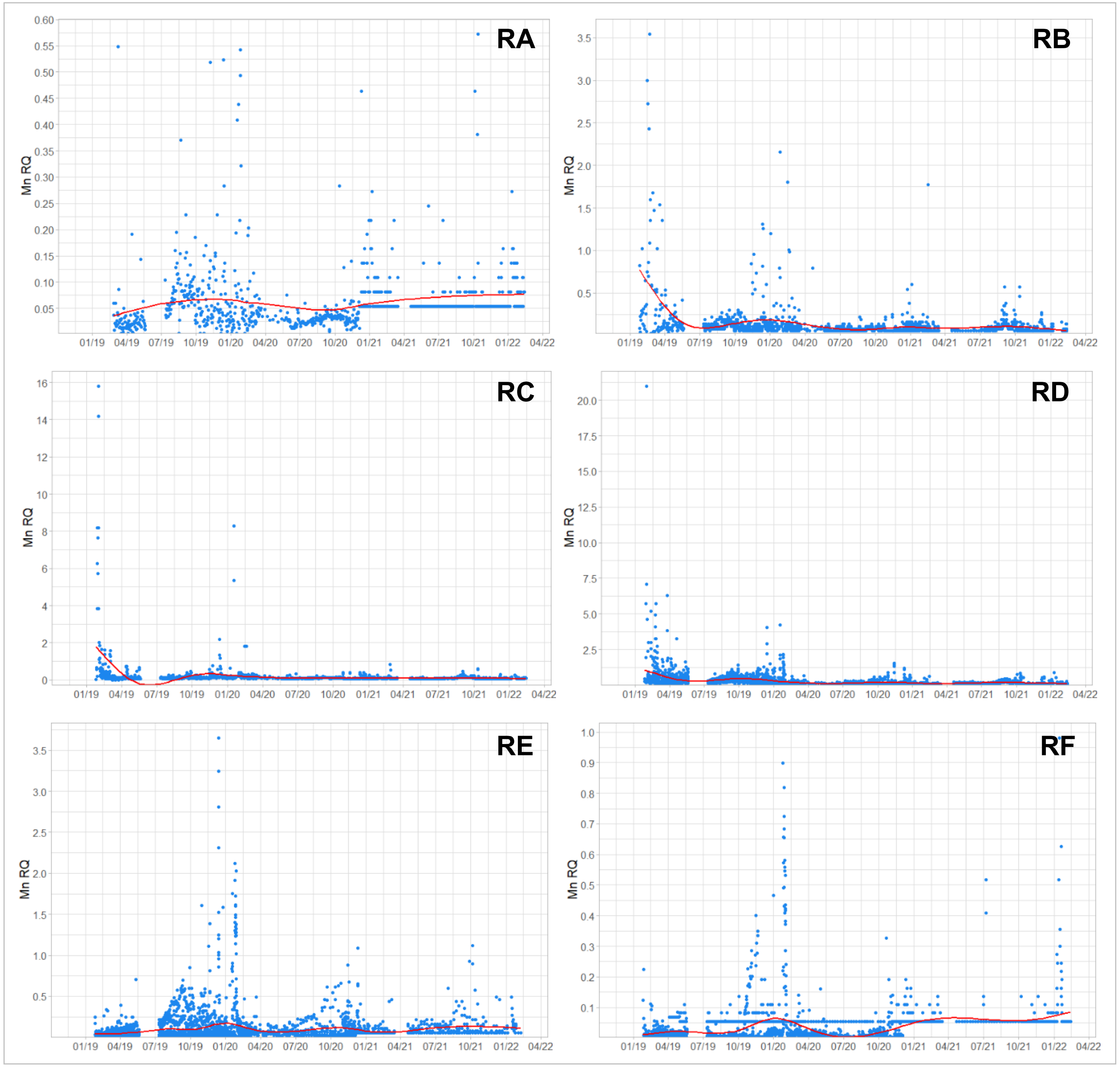
Source: author.

Figure 23 – Time series of Fe chronic RQs in Paraopeba River regions A to F



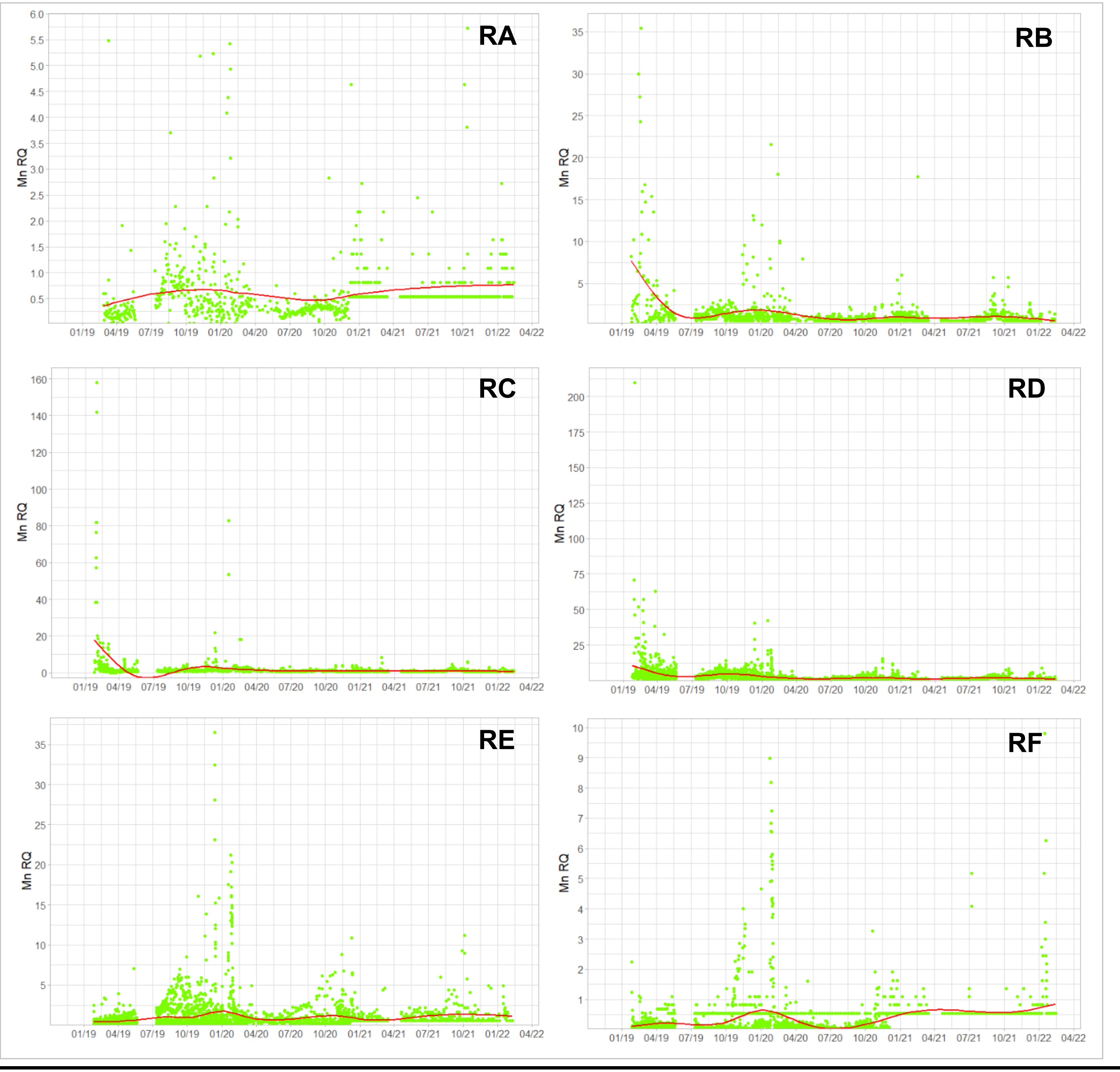
Source: author.

Figure 24 – Time series of Mn acute RQs in Paraopeba River regions A to F



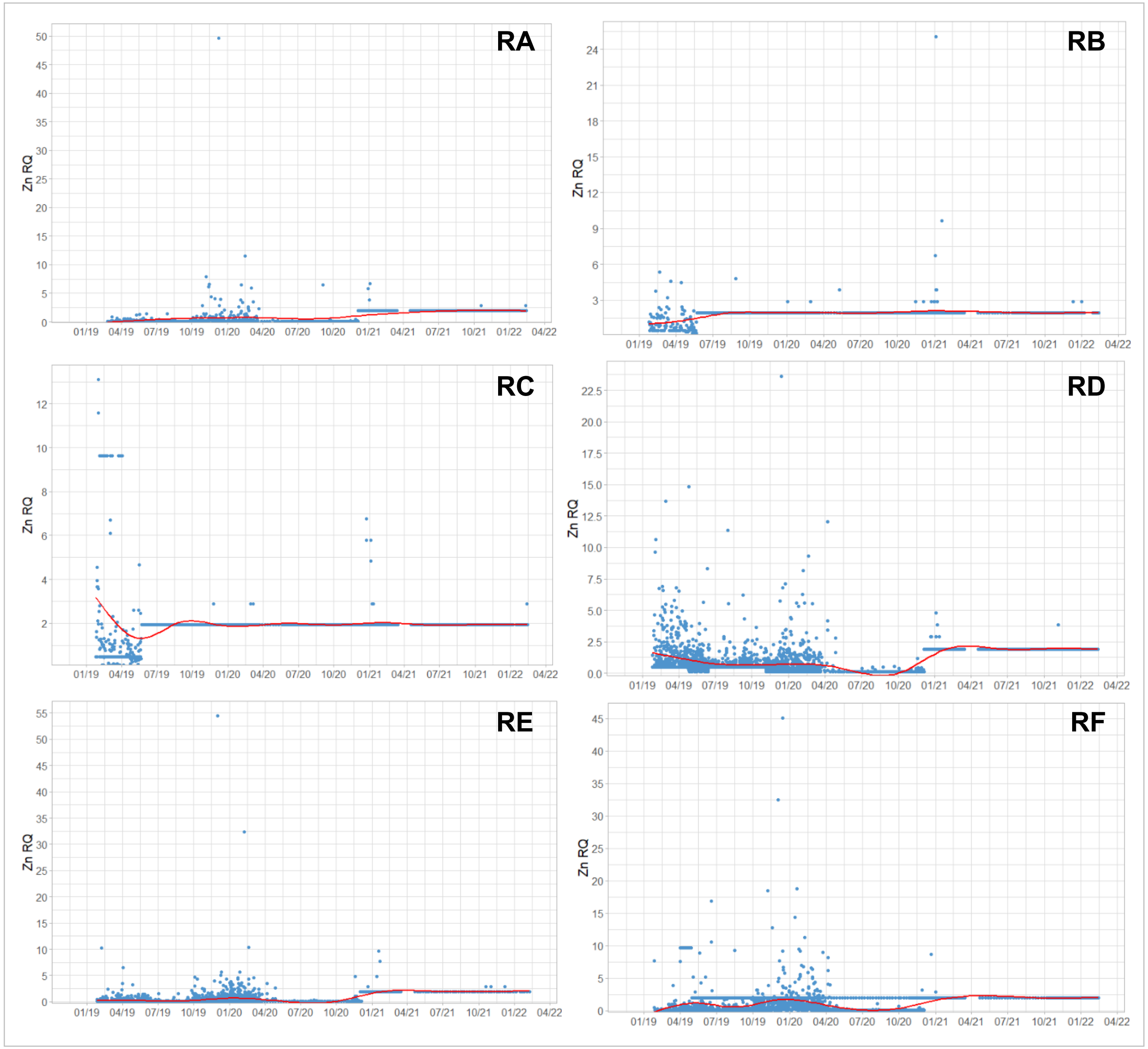
Source: author.

Figure 25 – Time series of Mn chronic RQs in Paraopeba River regions A to F



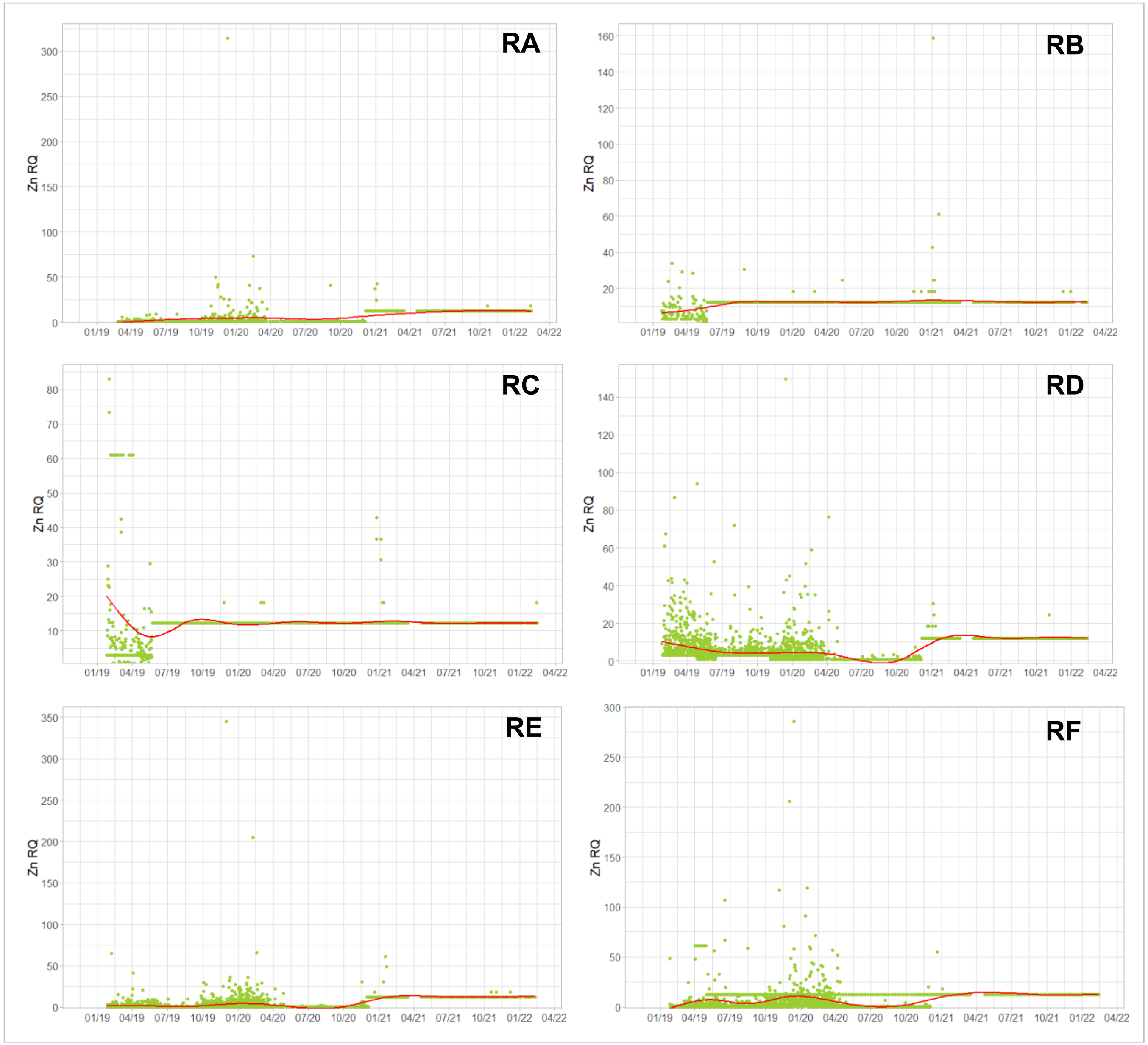
Source: author.

Figure 26 – Time series of Zn acute RQs in Paraopeba River regions A to F



Source: author.

Figure 27 – Time series of Zn chronic RQs in Paraopeba River Regions A to F



Source: author.

6 CONCLUSIONS

This study contributed to the knowledge field on ecotoxicology and risk assessment by proposing HC_5 and PNEC values for 14 metals based on SSD curves built for three trophic levels and acute and chronic effects. This is the first study to build such curves for each group and using at least seven species per trophic level, thus delivering solid results to be used by the scientific community and environmental agencies for risk assessment studies and to set legal standards which are protective of most aquatic organisms. The PNEC values proposed in this study should not replace ecotoxicity assays carried out in laboratory with environmental samples or be used as legal standards, but they might be used to indicate priority metals/metalloids and trophic groups, and to subside the development of new legislation directives and laboratory studies.

The smallest HC_5 and PNEC values were related to Ag, thus being the most toxic metal according to results reported by ecotoxicological assays. Based on acute and chronic effects of all the evaluated metals, invertebrates were the most sensitive group, while fishes were the most resistant. This indicates that in areas of known metal contamination and following events associated to contamination by metals, invertebrates should be the priority group for the conduction of ecotoxicological assays and biomonitoring, in order to measure impacts and evaluate the effectiveness of actions carried out to repair damage to aquatic life.

Based on HC_5 and PNEC values obtained in this study for all 14 metals, it was possible to evaluate current Brazilian and Minas Gerais state water quality standards associated to these compounds regarding their level of protection to the aquatic biota. The Brazilian legal limits for metals and metalloids were proposed more than 18 years ago and the Minas Gerais legal limits for these substances were not revised in the newest version of the legislation published in November 2022. These limits were higher than the PNEC values calculated in this study for more than 50% of cases. This indicates that legal limits would, in general, not be protective to the aquatic biota. For chronic effects, this protection level might be even smaller, since in 78% of PNECs proposed in this study were smaller than legal limits. Ag was the most serious case, since all the PNEC values were smaller than current legal limits. Besides Ag, based on legal limits, the aquatic biota might not be protected for chronic effects of Al, Cd, Cr, Fe, Mn, Ni, U and Zn. Thus, it is critical to rethink and revise legal limits, considering regional specificities and specific characteristics of each watershed.

In addition, this study also showed that bioavailability influences HC_5 values. So, it is critical to consider this aspect when setting standards and performing risk assessment studies in a local basis as it is done in developed regions. Although, this is still a challenge as there are no available tools to provide for conversion of bioavailability of all legislated metals and metalloids. Furthermore, the characteristics of water that mainly influence bioavailability, such as pH, DOC and hardness, change along the study area and time.

Moreover, a spatiotemporal evaluation of acute and chronic ecotoxicological risks to freshwater organisms along the Paraopeba River after the B1 dam collapse was carried out. The assessment revealed that from January 2019 to February 2022, monitoring areas located from 400 m upstream to 68,4 km downstream the collapse point were identified as the areas of highest risk to aquatic life, thus, requiring more efforts for water quality monitoring, including ecotoxicological assays, and measures for watershed restoration and aquatic life protection. These regions presented significantly highest risks than the other regions until the dry period of 2021, when risks became similar between upstream and downstream the dam, except for region RF.

The ecotoxicological risk assessment also allowed to identify that Fe was the metal that represented highest ecotoxicological risk to the aquatic biota in the Paraopeba River water after the B1 dam collapse, followed by Al. This was not surprising, since these metals are naturally present in the region soil and those were the main metals which composed the tailings. Peaks of RQs related to the dam collapse were verified for all the evaluated metals, except for As, Cd, Cr, Hg and Zn, for which it was not possible to make a direct association between the highest RQs values with the dam collapse due to the existence of various anthropic activities in the watershed, influence of tributaries and the date and region that the highest values occurred.

Despite absence of significant difference in risks associated to metals in the Paraopeba River water along time and space, this does not indicate that low risk values were achieved. The absence of significant differences in risks in regions located downstream the former dam in comparison to the upstream region does not mean lack of risk to the aquatic biota. After all, the upstream area is also highly impacted by anthropic activities.

7 RECOMENDATIONS

Based on results obtained in this study and limitations of the present study, the following suggestion are recommended for future works:

- To conduct laboratory assays with standardized conditions and native species, in order to build a more robust database for the definition of PNEC values from SSD curves;
- To evaluate the impact of considering bioavailability before constructing SSD curves, that is, to assure that responses from ecotoxicological assays reflect the bioavailable fraction, instead of correcting the HC_5 value to consider bioavailability, as it was done in the present study;
- To evaluate the impact of mixtures of metals in SSD curves so that they will reflect on HC_5 values;
- To assess the impact of the B1 tailings dam collapse in the evolution of risks of the Paraopeba River sediments;
- To evaluate the risks in the tributaries of the Paraopeba River and other locations of the watershed to verify the impacts of land use and human activities in RQs;
- To conduct an ecotoxicological risk evaluation with a database which comprises Paraopeba River water quality data obtained prior to dam collapse and for a longer period after the collapse.

REFERENCES

ADAMS, W.; BLUST, R.; DWYER, R.; MOUNT, D.; NORDHEIM, E.; RODRIGUEZ, P. H.; SPRY, D. Bioavailability assessment of metals in freshwater environments: A historical review. **Environmental Toxicology and Chemistry**, v. 39, n. 1, p. 48-55, 2020.

AGÊNCIA NACIONAL DE ÁGUAS E SANEAMENTO BÁSICO - ANA. **Relatório de segurança de barragens 2020**. Available at: < https://www.snisb.gov.br/relatorio-anual-de-seguranca-de-barragem>. Access in 08 Dec. 2021.

AGÊNCIA NACIONAL DE MINERAÇÃO - ANM. **Classificação nacional de barragens de mineração**. Available at: <https://app.anm.gov.br/SIGBM/Publico/ClassificacaoNacional Da Barragem>. Access in: 23 Feb. 2023.

ALDENBERG, T.; JAWORSKA, J. S. Uncertainty of the hazardous concentration and fraction affected for normal Species Sensitivity Distributions. **Ecotoxicology and Environmental Safety**, v. 46, p. 1–18, 2000.

ALHO, L. O. G.; GEBARA, R.C.; MANSANO, A.S.; ROCHA, G.S.; MELÃO, M.G.G. Individual and combined effects of manganese and chromium on a freshwater Chlorophyceae. **Environmental Toxicology and Chemistry**, v.0, n. 0, p.1-12, 2022.

ALMEIDA, V. O.; PEREIRA, T.C.B.; TEODORO, L.S.; ESCOBAR, M.; ORDOVÁS, C.J.; SANTOS, K.B.; WEILER, J.; BOGO, M.R.; SCHNEIDER, A.H. On the effects of iron ore tailings micro/nanoparticles in embryonic and larval zebrafish (*Danio rerio*). **Science of the Total Environment**, v. 759, p. 143456, 2021

ARAMBAWATTA-LEKAMGE, S. H.; PATHIRATNE, A.; RATHNAYAKE, I. V. N. Sensitivity of freshwater organisms to cadmium and copper at tropical temperature exposures: derivation of tropical freshwater ecotoxicity thresholds using species sensitivity distribution analysis. **Ecotoxicology and Environmental Safety,** v. 211, 2021.

ARMSTRONG, M.; PETTER, R.; PETTER, C. Why have so many tailings dams failed in recent years? **Resources Policy**, v. 63, p. 104412, 2019.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 12,648**: Aquatic ecotoxicology - Chronic toxicity - Test with algae (Chlorophyceae). Rio de Janeiro: ABNT, 2018.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 12,713**: Aquatic ecotoxicology — Acute toxicity — Test with *Daphnia* spp (Cladocera, Crustacea). Rio de Janeiro: ABNT, 2016.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 12,716**: Water - Acute toxicity test with fish - Part III - Flow through method - Method of test. Rio de Janeiro: ABNT, 1993.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 13,373**: Aquatic ecotoxicology - Chronic toxicity - Test method with *Ceriodaphnia* spp (Crustacea, Cladocera). Rio de Janeiro: ABNT, 2017.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 15,088**: Aquatic ecotoxicology - Acute toxicity - Test with fish (Cyprinidae). Rio de Janeiro: ABNT, 2016.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 15,411-1**: Aquatic ecotoxicology - Inhibitory effect on *Vibrio fischeri* bioluminenscence - Part 1: Method using freshly prepared bacterias. Rio de Janeiro: ABNT, 2021.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 15,411-2**: Ecotoxicology aquatic - Inhibitory effect on *Vibrio fischeri* bioluminenscence - Part 2: Method using liquid-dried bacterias. Rio de Janeiro: ABNT, 2021.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 15,411-3**: Ecotoxicology aquatic - Inhibitory effect on *Vibrio fischeri* bioluminenscence - Part 3: Method using freeze-dried bacterias. Rio de Janeiro: ABNT, 2011.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 15,469**: Aquatic Ecotoxicology – Collection, preservation and preparation of samples. Rio de Janeiro: ABNT, 2021.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **ABNT NBR 15,499**: Aquatic ecotoxicology - Short-term chronic toxicity - Test with fish. Rio de Janeiro: ABNT, 2016.

AZAM, S.; LI, Q. **Tailings dam failures: a review of the last one hundred years**. 2010. Available at: <https://ksmproject.com/wp-content/uploads/2017/08/Tailings-Dam-Failures-Last-100-years-Azam2010.pdf>. Access in: 30 Nov. 2021.

BASTIDAS-MARTÍNEZ, J.G.; CARVALHO, J. C.; CRISTHIANE, L. L.; FARIAS, M.M. Effects of Iron Ore Tailing on Performance of Hot-Mix Asphalt. **Journal of Materials in Civil Engineering**, v. 34, n. 1, 2022.

BELANGER, S. E.; CARR, G. J. SSDs revisited: part II - practical considerations in the development and use of application factors applied to Species Sensitivity Distributions. **Environmental Toxicology and Chemistry**, v. 38, n. 7, p. 1526–1541, 2019.

BELANGER, S.; BARRON, M.; CRAIG, P.; DYER, S.; GALAY-BURGOS, M.; HAMER, M.; MARSHALL, S.; POSTHUMA, L.; RAIMONDO, S.; WHITEHOUSE, P. Future needs and recommendations in the development of species sensitivity distributions: Estimating toxicity thresholds for aquatic ecological communities and assessing impacts of chemical exposures. **Integrated Environmental Assessment and Management**, v. 13, n. 4, p. 664–674, 2017.

BERTOLETTI, E. **Controle ecotoxicológico de efluentes líquidos no Estado de São Paulo**. 2013. Available at: <https://cetesb.sp.gov.br/wp-content/uploads/2015/06/manual-controle-ecotoxicologico-2013.pdf>. Access in: 26 Dec. 2021.

BESSER, J. M.; BRUMBAUGH, A.L.A.; POULTON, B.C.; SCHMITT, C.J.; INGERSOLL, C.G. Ecological impacts of lead mining on Ozark streams: Toxicity of sediment and pore water. **Ecotoxicology and Environmental Safety**, v. 72, n. 2, p. 516–526, 2009.

BEULAH, M.; CHANDRA, K.S.; MOHAN, M.K.; DEAN, I.C.; GAYATHRI, G. An experimental study on utilization of red mud and iron ore tailings in production of stabilized blocks. **Lecture Notes in Civil Engineering**, v. 72, p. 9–19, 2020.

BIO-MET. **bio-met bioavailability tool User Guide (version 5.1)**. 2021. Available at: <www.bio-met.net>. Access in: 25 Feb. 2023.

BRASIL. Conselho Nacional de Meio Ambiente – CONAMA. **Resolução CONAMA nº 357, de 17 de março de 2005**. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Brasília: CONAMA, 2005.

BRASIL. **Resolução CONAMA nº 430 de 13 de maio de 2011**. Dispõe sobre as condições e padrões de lançamento de efluentes, complementa e altera a Resolução nº 357, de 17 de março de 2005, do Conselho Nacional do Meio Ambiente - CONAMA. Brasília: CONAMA, 2011.

BRIX, K. V.; TEAR, L.; SANTORE, R. C.; CROTEAU, K.; DEFOREST, D. K. Comparative performance of multiple linear regression and Biotic Ligand Models for estimating the bioavailability of copper in freshwater. **Environmental Toxicology and Chemistry**, v. 40, n. 6, p. 1649–1661, 2021.

BUCH, A. C. SAUTTER, K.D.; MARQUES, E.D.; SILVA-FILHO, E.V. Ecotoxicological assessment after the world's largest tailing dam collapse (Fundão dam, Mariana, Brazil): effects on oribatid mites. **Environmental Geochemistry and Health**, v. 42, n. 11, p. 3575–3595, 2020.

BUCH, A. C.; NIEMEYER, J.C.; MARQUES, E.D.; SILVA-FILHO, E.V. Ecological risk assessment of trace metals in soils affected by mine tailings. **Journal of Hazardous Materials**, v. 403, p. 123852, 2021.

CANADIAN COUNCIL OF MINISTERS OF THE ENVIRONMENT - CCME. **Canadian Water Quality Guidelines for the protection of aquatic life**. Available at: <https://ccme.ca/en/current-activities/canadian-environmental-quality-guidelines>. Access in: 8 May 2022.

CIONEK, V. M.; ALVES, G.H.Z.; TÓFOLI, R.M.; RODRIGUES-FILHO, J.L.; DIAS, R.M. Brazil in the mud again: lessons not learned from Mariana dam collapse. **Biodiversity and Conservation –** Letter to the Editor, 2019.

COMBER, S.; GEORGES, K. **Tiered approach to the assessment of metal compliance in surface waters**. Bristol: Environment Agency, 2008. Available at: <www.wfduk.org>. Access in: 8 Jan. 2023.

CONNORS, K. A.; BEASLEY, A.; BARRON, M. G.; BELANGER, S. E.; BONNELL, M.; BRILL, J. L.; DE ZWART, D.; KIENZLER, A.; KRAILLER, J.; OTTER, R.; PHILLIPS, J. L.; EMBRY, M. R. **Creation of a curated aquatic toxicology database: EnviroTox**. Available at: <https://envirotoxdatabase.org/>. Access in: 28 Out. 2022.

CORDEIRO, M. C.; GARCIA, G. D.; ROCHA, A. M.; TSCHOEKE, D. A.; CAMPEÃO, M. E.; APPOLINARIO, L. R.; SOARES, A. C.; LEOMIL, L.; FROES, A.; BAHIENSE, L.; REZENDE, C. E.; DE ALMEIDA, M. G.; RANGEL, T. P.; DE OLIVEIRA, B. C. V.; DE ALMEIDA, D. Q. R.; THOMPSON, M. C.; THOMPSON, C. C.; THOMPSON, F. L. Insights on the freshwater microbiomes metabolic changes associated with the world's largest mining disaster. **Science of the Total Environment**, v. 654, p. 1209–1217, 2019.

COSTA, C. R.; OLIVI, P. A toxicidade em ambientes aquáticos: Discussão e métodos de avaliação. **Química Nova**, v. 31, n. 7, p. 1820–1830, 2008.

COSTA, C. R.; OLIVI, P.; BOTTA, C. M. R.; ESPINDOLA, E. L. G. A toxicidade em ambientes aquáticos: discussão e métodos de avaliação. **Química Nova**, v. 31, n. 7, p. 1820–1830, 2008.

COTA, G. E. M.; MAGALHÃES JÚNIOR, A. P. Panorama das barragens de rejeito de minério no Quadrilátero Ferrífero (MG) e suas implicações para a segurança hídrica da Região Metropolitana de Belo Horizonte-MG. **GeoTextos**, v. 17, n. 1, 2021.

DEL SIGNORE, A.; HENDRIKS, A.J.; LENDERS, H.J.B.; LEUVEN, R.E.S.W.; BREURE, A.M. Development and application of the SSD approach in scientific case studies for ecological risk assessment. **Environmental Toxicology and Chemistry**, v. 35, n. 9, p. 2149-2161, 2016.

DI LORENZO, T.; CIFONI, M.; FIACAS, B.; DI COCCIO, A.; GALASSI, D.M.P Ecological risk assessment of pesticide mixtures in the alluvial aquifers of central Italy: Toward more realistic scenarios for risk mitigation. **Science of the Total Environment**, v. 644, p. 161–172, 2018.

DO, T. M.; LAUE, J.; MATTSON, H.; JIA, Q. Numerical analysis of an upstream tailings dam subjected to pond filling rates. **Applied Sciences**, v. 11, n. 13, 2021.

DOMINGOS, L. M. B.; CASTILHOS, Z. C. Avaliação de riscos à saúde humana e ecológicos por rompimento da Barragem I da Vale em Brumadinho-MG. In: **Jornada do Programa de Capacitação Interna do CETEM, 9**. Rio de Janeiro: CETEM/MCTI, 2019.

EGOROVA, K. S.; ANANIKOV, V. P. Toxicity of metal compounds: knowledge and myths. **Organometallics**, v. 36, n. 21, p. 4071–4090, 2017.

EUROPEAN COMMISSION. **Common Implementation Strategy for the Water Framework Directive (2000/60/EC): Technical Guidance for Deriving Environmental Quality Standards. European Commission, Technical Report – 2011 – 055.** 2011. Available at: <http://ec.europa.eu/health/scientific_committees/experts/declarations/scheer_en>. Access in: 18 Jan. 2022.

EUROPEAN COMMISSION. **Technical guidance document on risk assessment - Part II**. 2003. Available at: <https://publications.jrc.ec.europa.eu/repository/handle/JRC23785>. Access in: 26 Apr. 2022.

EUROPEAN PARLIAMENT. **Directive 2008/105/EC of the European Parliament and of the Council 2008**. On environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Bruxelas: European Parliament, 2008.

EUROPEAN PARLIAMENT. **Directive 2013/39/EU of the European Parliament and of the Council**. Amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Bruxelas: European Parliament, 2013.

FERNANDES, G. W.; GOULART, F.F.; RANIERI, B.D.; COELHO, M.S.; DALES, K.; BOESCHE, N.; BUSTAMANTE, M.; CARVALHO, F.A.; CARVALHOP, D.C.; DIRZO, R.; FERNANDES, S.; GALETTI JR., P.M.; MILLA, V.E.G.; MIELKE, C.; RAMIREZ, J.L.; NEVES, A.; ROGASS, C.; RIBEIRO, S.P.; SCARIOT, A.; SOARES-FILHO, B. Deep into the mud: Ecological and socio-economic impacts of the dam breach in Mariana, Brazil**. Natureza e Conservação**, p. 35-45, 2016.

FERREIRA, D. B.; BARROSO, G. R.; DANTAS, M. S.; OLIVEIRA, K. L. de; CHRISTOFARO, C.; OLIVEIRA, S. C. Pluviometric patterns in the São Francisco River basin in Minas Gerais, Brazil. **Revista Brasileira de Recursos Hidricos**, v. 26, n.27, p. 1-13, 2021.

FOX, D. R.; VAN DAM, R.A.; FISHER, R.; BATLEY, G.E.; TILLMANNS, A.R.; THORLEY, J.; SCHWARTZ, C.J.; SPRY, D.J.; MCTAVISH, K. Recent developments in Species Sensitivity Distribution modeling. **Environmental Toxicology and Chemistry**, v. 40, n. 2, p. 293-308, 2021.

FREITAS, V. A. A.; BREDER, S.M.; SILVAS, F.P.C.; ROUSE, P.R.; OLIVEIRA, L.C.A. Use of iron ore tailing from tailing dam as catalyst in a fenton-like process for methylene blue oxidation in continuous flow mode. **Chemosphere**, v. 219, p. 328–334, 2019.

FUNDAÇÃO ESTADUAL DE MEIO AMBIENTE – FEAM; INSTITUTO ESTADUAL DE FLORESTAS – IEF; INSTITUTO MINEIRO DE GESTÃO DAS ÁGUAS – IGAM. **Caderno de 2 anos - Recuperação da bacia do rio Paraopeba após o rompimento das barragens da Vale, 2021**. Available at: <http://feam.br/recuperacao-ambiental-da-bacia-do-rio-paraopeba/-acoes-e-programas-de-recuperacao-ambiental-da-bacia-hidrografica-do-rio-paraopeba>. Access in: 8 Dec. 2021.

GABRIEL, F. A.; SILVA, A. G.; QUEIROZ, H. M.; FERREIRA, T. O.; HAUSER-DAVIS, R. A.; BERNARDINO, A. F. Ecological risks of metal and metalloid contamination in the Rio Doce estuary. **Integrated Environmental Assessment and Management**, v. 16, n. 5, p. 655–660, 2020.

GAO, P.; LI, Z.;GIBSON, M.; GAO, H.; Ecological risk assessment of nonylphenol in coastal waters of China based on Species Sensitivity Distribution model. **Chemosphere**, v. 104, p. 113–119, 2014.

GEBARA, R. C.; ALHO, L. de O. G.; ROCHA, G. S.; MANSANO, A. da S.; MELÃO, M. da G. G. Zinc and aluminum mixtures have synergic effects to the algae *Raphidocelis subcapitata* at environmental concentrations. **Chemosphere**, v. 242, p. 125231, 2020.

GLOTOV, V. E.; CHLACHULA, J.; GLOTOVA, L.P.; LITTLE, E. Causes and environmental impact of the gold-tailings dam failure at Karamken, the Russian Far East. **Engineering Geology**, v. 245, p. 236–247, 2018.

GOMES, L. C.; CHIPPARI-GOMES, A. R.; MIRANDA, T.O.; PEREIRA, T.M.; MERÇON, J.; DAVEL, V.C.; B. V. BARBOSA, B.V.; PEREIRA, A.C.H.; FROSSARD, A.; RAMOS, J. P. L. Genotoxicity effects on *Geophagus brasiliensis* fish exposed to Doce River water after the environmental disaster in the city of Mariana, MG, Brazil. **Brazilian Journal of Biology**, v. 79, n. 4, p. 659–664, 2019.

GREDELJ, A.; BARAUSSE, A.; GRECHI, L.; PALMERI, L. Deriving predicted no-effect concentrations (PNECs) for emerging contaminants in the river Po, Italy, using three approaches: Assessment factor, species sensitivity distribution and AQUATOX ecosystem modelling. **Environment International**, v. 119, p. 66–78, 2018.

GROSELL, M.; GERDES, R.; BRIX, K. v. Influence of Ca, humic acid and pH on lead accumulation and toxicity in the fathead minnow during prolonged water-borne lead exposure. **Comparative Biochemistry and Physiology – Part C**, v. 143, p. 473–483, 2006.

GUAN, B.; GUO, L.; GIBSON, M.; LI, Z. The derivation of water quality criteria for bisphenol a for the protection of marine species in China. **Water Quality Research Journal**, v. 53, n. 3, p. 156–165, 2018.

HARFORD, A. J.; MOONEY, T. J.; TRENFIELD, M. A.; VAN DAM, R. A. Manganese toxicity to tropical freshwater species in low hardness water. **Environmental Toxicology and Chemistry**, v. 34, n. 12, p. 2856–2863, 2015.

HAYASHI, T. I.; KASHIWAGI, N. A Bayesian approach to probabilistic ecological risk assessment: Risk comparison of nine toxic substances in Tokyo surface waters. **Environmental Science and Pollution Research**, v. 18, n. 3, p. 365–375, 2011.

HIKI, K.; IWASAKI, Y. Can we reasonably predict chronic Species Sensitivity Distributions from acute Species Sensitivity Distributions? **Environmental Science and Technology**, v. 54, n. 20, p. 13131–13136, 2020.

HU, W.; XIN, C.L.; LI, Y.; ZHENG, Y.S.; ASCH, T.W.J.; MCSAVENEY, M. Instrumented flume tests on the failure and fluidization of tailings dams induced by rainfall infiltration. **Engineering Geology**, v. 294, p. 106401, 2021.

HUI, S.; CHENGLIAN, F.; HONG, H.; FENGCHANG, W. The correlation discussion between aluminum toxicity to aquatic organisms and water hardness. **Asian Journal of Ecotoxicology**, v. 11, n. 1, p. 141–152, 2016.

INSTITUTO BRASILEIRO DE MINERAÇÃO – IBRAM. **Faturamento Setor Mineral - 3 º Trimestre 2022**. Available at: <https://ibram.org.br/noticia/faturamento-do-setor-mineral-sobe-33-no-3o-tri-em-relacao-ao-2o-tri-mas-decai-30-em-relacao-ao-3o-tri-de-2021/>. Access in: 10 Feb. 2023.

INSTITUTO BRASILEIRO DE MINERAÇÃO – IBRAM. **Gestão e manejo de rejeitos da mineração**. Brasília: IBRAM, 2016.

INSTITUTO DE PESQUISAS TECNOLÓGICAS – IPT. **Rejeitos de mineração**. 2016. Available at: <https://www.ipt.br/noticias_interna.php?id_noticia=1043>. Access in: 26 Nov. 2021.

INSTITUTO MINEIRO DE GESTÃO DAS ÁGUAS – IGAM. **Avaliação da qualidade das águas e sedimentos do Rio Paraopeba.** 2021. Available at: <http://feam.br/recuperacao-ambiental-da-bacia-do-rio-paraopeba/-acoes-e-programas-de-recuperacao-ambiental-da-bacia-hidrografica-do-rio-paraopeba>. Access in: 8 Dec. 2021.

INSTITUTO MINEIRO DE GESTÃO DAS ÁGUAS – IGAM. **Plano diretor de recursos hídricos da bacia hidrográfica do Rio Paraopeba - SF3 resumo executivo**. 2019a. Available at: <http://portalinfohidro.igam.mg.gov.br/images/Resumo_Executivo.pdf>. Access in: 28 Dec. 2021.

INSTITUTO MINEIRO DE GESTÃO DAS ÁGUAS – IGAM. **Informativo Especial – Avaliação da série histórica entre 2000 e 2018. Informativo dos parâmetros de qualidade das águas nos locais monitorados ao longo do Rio Paraopeba antes do desastre na barragem B1 no complexo da Mina Córrego Feijão da Mineradora Vale/SA no município de Brumadinho – Minas Gerais**. 2019b. Available at: <http://www.meioambiente.mg.gov.br/images/stories/2019/DESASTRE_BARRAGEM_B1/informativos_qualidade_agua/Informativo_Especial__Serie_Hist%C3%B3rica_2000_a_2018_140219.pdf>. Access in: 11 Apr. 2023.

ISLAM, K.; MURAKAMI, S. Global-scale impact analysis of mine tailings dam failures: 1915–2020. **Global Environmental Change**, v. 70, p. 102361, 2021.

IWASAKI, Y.; KOTANI, K.; KASHIWADA, S.; MASUNAGA, S. Does the choice of NOEC or EC_{10} affect the hazardous concentration for 5% of the species? **Environmental Science and Technology**, v. 49, n. 15, p. 9326–9330, 2015.

JACOB, R. S. **Avaliação da contaminação aquática por fármacos utilizando análises ecotoxicológicas**. 2017. Dissertation (Master's in Sanitation, Environment and Water Resources) - Universidade Federal de Minas Gerais, Belo Horizonte, 2017, 149 p.

JOHNSON, I.; SOROKIN, N.; ATKINSON, C.; RULE, K.; HOPE, S.-J. **Preconsultation report: proposed EQS for Water Framework Directive Annex VIII substances: Iron (total dissolved)**. Bristol: Environmental Agency, 2007. Available at: <www.environment-agency.gov.uk>. Access in: 25 Feb. 2023.

KALSNES, B.; JOSTAD, H.P.; NADIM, F.; HAUGE, A.; DUTRA, A.; MUXFELDT, A. Tailings dam stability. In: **4th World Lanslide Forum**. Congress proceedings. Lhubljana: Springer International Publishing, Advancing Culture of Living with Landslides, p. 1173–1180, 2017.

KIM, D.; CUI, R.; MOON, J.; KWAK, J.I.; KIM, S.W.; KIM, D.; AN, Y. Estimation of the soil hazardous concentration of methylparaben using a species sensitivity approach. **Environmental Pollution**, v. 242, p. 1002–1009, 2018.

KOSSOFF, D.; DUBBIND, W.E.; ALFREDSSON, M.; EDWARDS, S.J.; MACKLIN, M.G.; HUDSON-EDWARS, K.A. Mine tailings dams: Characteristics, failure, environmental impacts, and remediation. **Applied Geochemistry**, v. 51, p. 229–245, 2014.

KWAK, J.; LEE, T.; SEO, H.; KIM, D.; CUI, R.; AN, Y. Ecological risk assessment for perfluorooctanoic acid in soil using a species sensitivity approach. **Journal of Hazardous Materials**, v. 382, 2020.

LABANDA, N. A.; SOTTILE, M.G.; CUETO, I.A.; SFRISO, A.O. Screening of seismic records to perform time-history dynamic analyses of tailings dams: A power-spectral based approach. **Soil Dynamics and Earthquake Engineering**, v. 146, p. 106750, 2021.

LATHOURI, M.; KORRE, A. Temporal assessment of copper speciation, bioavailability and toxicity in UK freshwaters using chemical equilibrium and biotic ligand models: Implications for compliance with copper environmental quality standards. **Science of the Total Environment**, v. 538, p. 385–401, 2015.

LEPPER, P.; SOROKIN, N.; MAYCOCK, D.; CRANE, M.; ATKINSON, C.; HOPE, S.J.; COMBER, S. **Preconsultation report: Proposed EQS for Water Framework Directive annex VIII substances: arsenic (total dissolved)**. Bristol: Environment Agency, 2007. Available at: <www.environment-agency.gov.uk>. Access in: 26 Feb. 2023.

LIMA, J. C. S.; GAZONATO NETO, A.J.; ANDRADE, D.P.; FREITAS, E.C.; MOREIRA, R.A.; MIGUEL, M.; DAAM, M.A.; ROCHA, O. Acute toxicity of four metals to three tropical aquatic invertebrates: The dragonfly Tramea cophysa and the ostracods *Chlamydotheca* sp. and *Strandesia trispinosa*. **Ecotoxicology and Environmental Safety**, v. 180, p. 535–541, 2019.

LYU, Z.; CHAI, J.; XU, Z.; QIN, Y.; CAO, J. A comprehensive review on reasons for tailings dam failures based on case history. **Advances in Civil Engineering**, v. 2019, p. 1-18, 2019.

MAGALHÃES, D. P.; FERRÃO FILHO, A. S. A ecotoxicologia como ferramenta para o biomonitoramento de ecossistemas aquáticos. **Oecologia Brasiliensis**, v. 12, n. 3, p. 355–381, 2008.

MAGALHÃES, D. P.; MARQUES, M. R. C.; BAPTISTA, D. F.; BUSS, D. F. Metal bioavailability and toxicity in freshwaters. **Environmental Chemistry Letters**, v. 13, p. 69-87, 2015.

MARKS, B.; PETERS, A.; MCGOUGH, D. Aquatic environmental risk assessment of manganese processing industries. **NeuroToxicology**, v. 58, p. 187-193, 2017.

MARUTHUPANDIAN, S.; CHALIASOU, A.; KANELLOPOULOS, A. Recycling mine tailings as precursors for cementitious binders – Methods, challenges and future outlook. **Construction and Building Materials**, v. 312, p. 125333, 2021.

MAYCOCK, D.; PETERS, A.; MERRINGTON, G.; CRANE, M. **Proposed EQS for Water Framework Directive annex VIII substances: Zinc (For consultation)**. Oxfordshire: Environmental Agency, 2012. Available at: <www.wfduk.org>. Access in: 26 Feb. 2023.

MAYCOCK. D.; SOROKIN, N.; ATKINSON, C.; RULE, K.; CRANE, M. **Proposed EQS for Water Framework Directive annex VIII substances: Chromium(VI) and chromium(III) (dissolved)**. Bristol: Environment Agency, 2007. Available at: <www.environment-agency.gov.uk>. Access in: 26 Feb. 2023.

MEBANE, C. A. **Bioavailability and toxicity models of copper and zinc to freshwater life: The state of the science and alternatives for water quality criteria**. Boise Idaho: US Geological Survey, 2022. Available at: <https://osf.io/smynf>. Access in: 10 Feb. 2023.

MEBANE, C. A.; CHOWDHURY, M. J.; DE SCHAMPHELAERE, K. A. C.; LOFTS, S.; PAQUIN, P. R.; SANTORE, R. C.; WOOD, C. M. Metal bioavailability models: Current status, lessons learned, considerations for regulatory use, and the path forward. **Environmental Toxicology and Chemistry**, v. 39, n. 1, p. 60-84, 2020b.

MEBANE, C. A.; SCHMIDT, T. S.; MILLER, J. L.; BALISTRIERI, L. S. Bioaccumulation and toxicity of cadmium, copper, nickel, and zinc and their mixtures to aquatic insect communities. **Environmental Toxicology and Chemistry**, v. 39, n. 4, p. 812–833, 2020a.

MENDES, L. B.; MELLO, F.A.; CHAGAS, K.A.; CAMPELO, R.P.M.; MEDEIROS, L.C.C.; SMITH, R.E.W.; FURLEY, T.H. Ecotoxicological assessment of the Doce River surface water after the Fundão Dam collapse. **Integrated Environmental Assessment and Management**, v. 16, n. 5, p. 608–614, 2020.

MINAS GERAIS. Conselho Estadual de Política Ambiental – COPAM e Conselho Estadual de Recursos Hídricos do Estado de Minas Gerais – CERH. **Deliberação Normativa Conjunta COPAM/CERH n° 01, de 5 de maio de 2008**. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelec e as condições e padrões de lançamento de efluentes, e dá outras providências. Belo Horizonte: COPAM/CERH, 2008.

MINAS GERAIS. Conselho Estadual de Política Ambiental – COPAM e Conselho Estadual de Recursos Hídricos do Estado de Minas Gerais – CERH. **Deliberação Normativa Conjunta COPAM/CERH n° 08, de 21 de novembro de 2022**. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelec e as condições e padrões de lançamento de efluentes, e dá outras providências. Belo Horizonte: COPAM/CERH, 2022.

MINAS GERAIS. Conselho Estadual de Política Ambiental – COPAM. **Deliberação Normativa COPAM n° 14, de 28 de dezembro de 1995**. Dispõe sobre o enquadramento das águas da Bacia do Rio Paraopeba. Belo Horizonte: COPAM, 1995.

MOURA, J. A. S.; SOUZA-SANTOS, L. P. Environmental risk assessment (ERA) of pyriproxyfen in non-target aquatic organisms. **Aquatic Toxicology**, v. 222, p. 105448, 2020.

NATH, A.; DE, P.; ROY, K. QSAR modelling of inhalation toxicity of diverse volatile organic molecules using No Observed Adverse Effect Concentration (NOAEC) as the endpoint. **Chemosphere**, v. 287, p. 131954, 2022.

NYS, C.; VAN REGENMORTEL, T.; JANSSEN, C. R.; OORTS, K.; SMOLDERS, E.; DE SCHAMPHELAERE, K. A. C. A framework for ecological risk assessment of metal mixtures in aquatic systems. **Environmental Toxicology and Chemistry**, v. 37, n. 3, p. 623–642, 2018.

PAN, Y.; CHEN, M.; WANG, X.; CHEN, Y.; DONG, K. Ecological risk assessment and source analysis of heavy metals in the soils of a lead-zinc mining watershed area. **Water**, v. 15, n. 113, p. 1-19, 2023.

PANDEY, L. K.; LAVOIE, I.; MORIN., S.; DEPUYDT, S.; LYU, J.; LEE, H.; JUNG, J.; UEOM, D.H.; HAN, T.; PARK, J. Towards a multi-bioassay-based index for toxicity assessment of fluvial waters. **Environmental Monitoring and Assessment**, v. 191, n. 2, 2019.

PARENTE, C. E. T.; LINO, A.S.; CARVALHO, G.O.; PIZZOCHERO, A.C.; AZEVEDO-SILVA, C.E.; FREITAS, M.O.; TEIXEIRA, C.; MOURA, R.L.; FERREIRA FILHO, V.J.M.; MALM, O. First year after the Brumadinho tailings' dam collapse: Spatial and seasonal variation of trace elements in sediments, fishes and macrophytes from the Paraopeba River, Brazil. **Environmental Research**, v. 193, p. 110526, 2021.

PARK, J.; KIM, S. D. Derivation of Predicted No Effect Concentrations (PNECs) for heavy metals in freshwater organisms in Korea using Species Sensitivity Distributions (SSDs). **Minerals**, v. 10, n. 8, p. 1–15, 2020.

PETERS, A.; CRANE, M.; MAYCOCK, D.; MERRINGTON, G.; SIMPSON, P. **Proposed EQS for Water Framework Directive annex VIII substances: Manganese (bioavailable) (For Consultation)**. Oxfordshire: Environmental Agency, 2010. Available at: <www.wfduk.org>. Access in: 26 Feb. 2023.

PINHO, M. Q.; FILHO, W. L. de O. Large strain consolidation analyses of fine tailings disposal in mining pits. **Revista Escola de Minas**, v. 73, n. 3, p. 411–419, 2020.

PLATAFORMA BRUMADINHO. **Infraestrutura de Dados Espaciais**. Available at: < http://ide.projetobrumadinho.ufmg.br/>. Access in: 24 Jan. 2023.

PORSANI, J. L.; DE JESUS, F. A. N.; STANGARI, M. C. GPR survey on an iron mining area after the collapse of the tailings Dam I at the Córrego do Feijão mine in Brumadinho-MG, Brazil. **Remote Sensing,** v. 11, n. 7, p. 1-13, 2019.

POSTHUMA, L.; SUTER II, G. W.; TRASS, T. P. **Species Sensitivity Distributions in ecotoxicology**. 1st Edition. New York: Lewis Publishers, 2002.

QUADRA, G. R.; ROLAND, F.; BARROS, N.; MALM, O.; LINO, A.S.; ZEEDO, G.M.; THOMAZ, J,R.; ANDRADE-VEIRA, L.F.; PRAÇA-FONTES, M.M.; ALMEIDA, R.M.; MENDONÇA, R.F.; CARDOSO, S.J.; GUIDA, Y.S.; CAMPOS, J.M.S. Far-reaching cytogenotoxic effect of mine waste from the Fundão dam disaster in Brazil. **Chemosphere**, v. 215, p. 753–757, 2019.

QUEIROZ, H. M. NÓBREGA, G.N.; FERREIRA, T.O.; ALMEIDA, L.S.; ROMERO, T.B.; SATELLA, S.T.; BERNARDINO, A.F.; OTERO, X.L. The Samarco mine tailing disaster: A possible time-bomb for heavy metals contamination? **Science of the Total Environment**, v. 637–638, p. 498–506, 2018.

RAND, G. M; PETROCELLI, S.R. **Fundamental of aquatic toxicology**. Washington: Hemisphere Publishing Corporation, 1985, 666p.

RAZAK, M. R.; ARIS, A.Z.; ZAKARIA, N.A.C.; WEE, S.Y.; ISMAIL, N.A.H. Accumulation and risk assessment of heavy metals employing species sensitivity distributions in Linggi River, Negeri Sembilan, Malaysia. **Ecotoxicology and Environmental Safety**, v. 211, p. 111905, 2021.

REIS, E. O.; SANTOS, L. V. S.; LANGE, L. C. Prioritization and environmental risk assessment of pharmaceuticals mixtures from Brazilian surface waters. **Environmental Pollution**, v. 288, p. 117803, 2021.

REISS, F.; KIEFER, N.; NOLL, M.; KALKHOF, S. Application, release, ecotoxicological assessment of biocide in building materials and its soil microbial response. **Ecotoxicology and Environmental Safety**, v. 224, p. 112707, 2021.

RIBA, I.; DELVALLS, T.A.; REYNOLDSON, T.; MILANI, D. Sediment quality in Rio Guadiamar (SW, Spain) after a tailing dam collapse: Contamination, toxicity and bioavailability. **Environment International**, v. 32, n. 7, p. 891–900, 2006.

RICO, M.; BENITO, G.; DÍEZ-HERRERO, A. Floods from tailings dam failures. **Journal of Hazardous Materials**, v. 154, p. 79–87, 2008.

ROTTA, L. H.S.; ALCÂNTARA, E.; PARK, E.; NEGRI, R.G.; LIN, Y.N.; BERNARDO, N.; MENDES, T.S.G.; SOUZA FILHO, C.R. The 2019 Brumadinho tailings dam collapse: Possible cause and impacts of the worst human and environmental disaster in Brazil. **International Journal of Applied Earth Observation and Geoinformation**, v. 90, p. 102119, 2020.

SCHOENBERGER, E. Environmentally sustainable mining: The case of tailings storage facilities. **Resources Policy**, v. 49, p. 119–128, 2016.

SCHVARTSMAN. **Intoxicações agudas**. 4th edition. São Paulo: Sarvier, 1991, 355 p.

SEGURA, F. R.; NUNES, E.A.; PANIZ, F.P.; PULELI, A.C.C.; RODRIGUES, G.B.; BRAGA, G.U.L.; PREDREIRA FILHO, W.R.; BARBOSA JR., F.; CERCHIARO, G.; SILVA, F.F.; BATISTA, B.L. Potential risks of the residue from Samarco's mine dam burst (Bento Rodrigues, Brazil). **Environmental Pollution**, v. 218, p. 813–825, 2016.

SERVIÇO GEOLÓGICO DO BRASIL - CPRM. **Monitoramento especial da bacia do Rio Paraopeba - Relatório 1: monitoramento hidrológico e sedimentológico 2019**. 2019. Available at: < https://rigeo.cprm.gov.br/handle/doc/21799>. Access in 10 Jan. 2022.

SHARMA, N.; SODHI, K. K.; KUMAR, M.; SINGH, D. K. Heavy metal pollution: Insights into chromium eco-toxicity and recent advancement in its remediation. **Environmental Nanotechnology, Monitoring and Management**, v. 15, p. 100388, 2021.

SHRESTHA, S.; KAZAMA, F. Assessment of surface water quality using multivariate statistical techniques: A case study of the Fuji river basin, Japan. **Environmental Modelling and Software**, v. 22, p. 464–475, 2007.

SISTEMA ESTADUAL DE MEIO AMBIENTE E RECURSOS HÍDRICOS - SISEMA. **Infraestrutura de Dados Espaciais do Sistema Estadual de Meio Ambiente e Recursos Hídricos**. Belo Horizonte: IDE-Sisema, 2023. Available at: < idesisema.meioambiente.mg.gov.br>. Access in: 26 Jan. 2023.

SOARES, A. L. C.; DUARTE, S.F.; GOMES, L.N.L.; OLIVEIRA, S.C. Impacto do rompimento da barragem de rejeitos de minério de ferro da mina do Feijão, em Brumadinho, quanto ao uso e à cobertura do solo e à qualidade das águas superficiais do Rio Paraopeba. **Revista UFMG**, v. 27, n. 2, p. 356–381, 2020.

SOARES, A. L. C.; OLIVEIRA, S. C.; GOMES, L. N. G. Influência das atividades antrópicas e do rompimento da Barragem I, da mina Córrego do Feijão, na qualidade da água da bacia hidrográfica do Rio Paraopeba. **Revista Mineira de Recursos Hídricos**, v. 3, p. 1–35, 2022.

SOARES, A. L. C.; PINTO, C. C.; OLIVEIRA, S. C. Impacts of anthropogenic activities and calculation of the relative risk of violating surface water quality standards established by environmental legislation: A case study from the Piracicaba and Paraopeba River basins, Brazil. **Environmental Science and Pollution Research**, v. 27, p. 14085–14099, 2020.

SORGOG, K.; KAMO, M. Quantifying the precision of ecological risk: Conventional assessment factor method vs. species sensitivity distribution method. **Ecotoxicology and Environmental Safety**, v. 183, p. 109494, 2019.

SOUZA, T. S.; BARONE, L.S.F.; LACERDA, D.; VERGILIO, C.S.; OLIVEIRA, B.C.V.; ALMEIDA, M.G.; THOMPSON, F.; REZENDE, C.E. Cytogenotoxicity of the water and sediment of the Paraopeba River immediately after the iron ore mining dam disaster (Brumadinho, Minas Gerais, Brazil). **Science of the Total Environment**, v. 775, p. 145193, 2021.

SPILSBURY, F. D.; WARNE, M. S. J.; BACKHAUS, T. Risk assessment of pesticide mixtures in Australian rivers discharging to the Great Barrier Reef. **Environmental Science and Technology**, v. 54, n. 22, p. 14361–14371, 2020.

TERAMOTO, E. H.; GEMEINER, H.; ZANATTA, M.B.T.; MENEGÁRIO, A.A.; CHANG, H.K. Metal speciation of the Paraopeba River after the Brumadinho dam failure. **Science of the Total Environment,** v. 757, p. 143917, 2021.

THOMPSON, F.; OLIVEIRA, B.C.; CORDEIRO, M.C.; MASI, BRUNO, P.; RANGEL, T.P.; PAZ, P.; FREITAS, T.; LPES, G.; SILVA. B.S.; CABRASL, A.S.; SOARES, M.; LACERDA, D.; VERGILIO, C.S.; LOPES-FERREIRA, M.; LIMA, C.; THOMPSON, C.; REZENDE, C.E. Severe impacts of the Brumadinho dam failure (Minas Gerais, Brazil) on the water quality of the Paraopeba River. **Science of the Total Environment**, v. 705, p.135914, 2020.

UMBUZEIRO, G. A. The need of scientific based regulations of chemicals in water: A proposal for Brazil. **Applied Research in Toxicology**, v. 1, p. 48–49, 2015.

UMBUZEIRO, G. A.; KUMMROW, F.; REI, F. F. C. Toxicologia, padrões de qualidade de água e a legislação. **InterfacEHS Revista de Gestão Integrada em Saúde do Trabalho e Meio Ambiente,** v. 5, n. 1, p. 1–15, 2010.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY – US EPA. **ECOTOX Knowledgebase**. 2022. Available at: < https://cfpub.epa.gov/ecotox/explore.cfm>. Access in 25 Feb. 2022.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY – US EPA. **National Recommended Water Quality Criteria**. 2023. Available at: <https://epa.gov/wqc/forms/contact-us-about-water-quality-criteria>. Access in 09 Jan 2023.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY – US EPA. **Using toxicity tests in ecological risk assessment**. 1994. Available at: <https://www.epa.gov/sites/default/files/2015-09/documents/v2no1.pdf>. Access in: 12 Dec. 2021.

UNIVERSIDADE FEDERAL DE OURO PRETO – UFOP. **Base de Dados – Quadrilátero Ferrífero 2050**. Available at: <https://qfe2050.ufop.br/bases-geologicas>. Access in: 16 Feb. 2023.

VÄÄNÄNEN, K.; LEPPÄNEN, M. T.; CHEN, X. P.; AKKANEN, J. Metal bioavailability in ecological risk assessment of freshwater ecosystems: From science to environmental management. **Ecotoxicology and Environmental Safety**, v. 147, p. 430-446, 2018.

VALE. **Plano de Reparação Socioambiental da Bacia do Rio Paraopeba – Rompimento das barragens B1, B4 e B4-A do complexo Paraopeba II da Mina Córrego do Feijão**. Capítulo 2. Volume 3 – Caracterização socioambiental pós-rompimento. Brumadinho: Arcadis,

2022. Available at: <https://www.mg.gov.br/pro-brumadinho/pagina/reparacao-brumadinho-plano-de-recuperacao-socioambiental-versao-preliminar>. Access in: 11 abr. 2023.

VALE. **Reparação e Desenvolvimento - Listas Atualizadas**. Available at: <https://saladeimprensa.vale.com/pt/reparacao>. Access in: 25 Jan. 2023.

VAN REGENMORTEL, T.; NYS, C.; JANSSEN, C. R.; LOFTS, S.; DE SCHAMPHELAERE, K. A. C. Comparison of four methods for bioavailability-based risk assessment of mixtures of Cu, Zn, and Ni in freshwater. **Environmental Toxicology and Chemistry**, v. 36, n. 8, p. 2123–2138, 2017.

VAN VLAARDINGEN, P. L. A.; TRAAS, T.P. WINTERSEN, A.M.; ALDENBERG, T. **ETX 2.0 A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data**. Bilthoven: National Institute for Public Health and the Environment, 2014. Available at: <https://www.rivm.nl/bibliotheek/rapporten/601501028.html> Access in: 26 Feb. 2022.

VERGILIO, C. S.; LACERDA, D.; OLIVEIRA, B.C.V.; SARTORI, E.; CAMPOS, G.M.C.; PEREIRA, A.L.S.; AGUAR, D.B.; SOUZA, T.S.; ALMEIDA, M.G.; THOMPSON, F.; REZENDE, C.E. Metal concentrations and biological effects from one of the largest mining disasters in the world (Brumadinho, Minas Gerais, Brazil). **Scientific Reports Nature Research**, v. 10, n. 1, p. 1–12, 2020.

VERGILIO, C. S.; LACERDA, D.; SOUZA, T.S.; OLIVEIRA, B.C.V.; FIORESEI, V.S.; SOUZA, V.V.; RODRIGUES, G.R.; BARBOSA, M.K.A.M.; SARTORI, E.; RANGEL, T.P.; ALMEIDA, D.Q.R.; ALMEIDA, M.G.; THOMPSON, F.; REZENDE, C.E. Immediate and long-term impacts of one of the worst mining tailing dam failure worldwide (Bento Rodrigues, Minas Gerais, Brazil). **Science of the Total Environment**, v. 756, p. 143697, 2021.

WANG, Z.; HO, K. K. Y.; ZHOU, G. J.; YEUNG, K. W. Y.; LEUNG, K. M. Y. Effects of silver and zinc on tropical freshwater organisms: Implications on water quality guidelines and ecological risk assessment. **Chemosphere**, v. 225, p. 897–905, 2019.

WANG, Z.; KWOK, K. W. H.; LEUNG, K. M. Y. Comparison of temperate and tropical freshwater species' acute sensitivities to chemicals: An update. **Integrated Environmental Assessment and Management**, v. 15, n. 3, p. 352–363, 2019.

WATER FRAMEWORK DIRECTIVE - WFD. **Metal Bioavailability Assessment Tool (M-BAT)**. Stirling: Water Framework Directive – United Kingdom Advisory Group, 2014. Available at: <www.wfduk.org>. Access in: 26 Feb. 2023.

WEBER, A. A.; SALES, C.F.; FARIA, F.S.; MELO, R.M.C.; BAZZOLI, N.; RIZZO, E. Effects of metal contamination on liver in two fish species from a highly impacted neotropical river: A case study of the Fundão dam, Brazil. **Ecotoxicology and Environmental Safety**, v. 190, p. 110165, 2020.

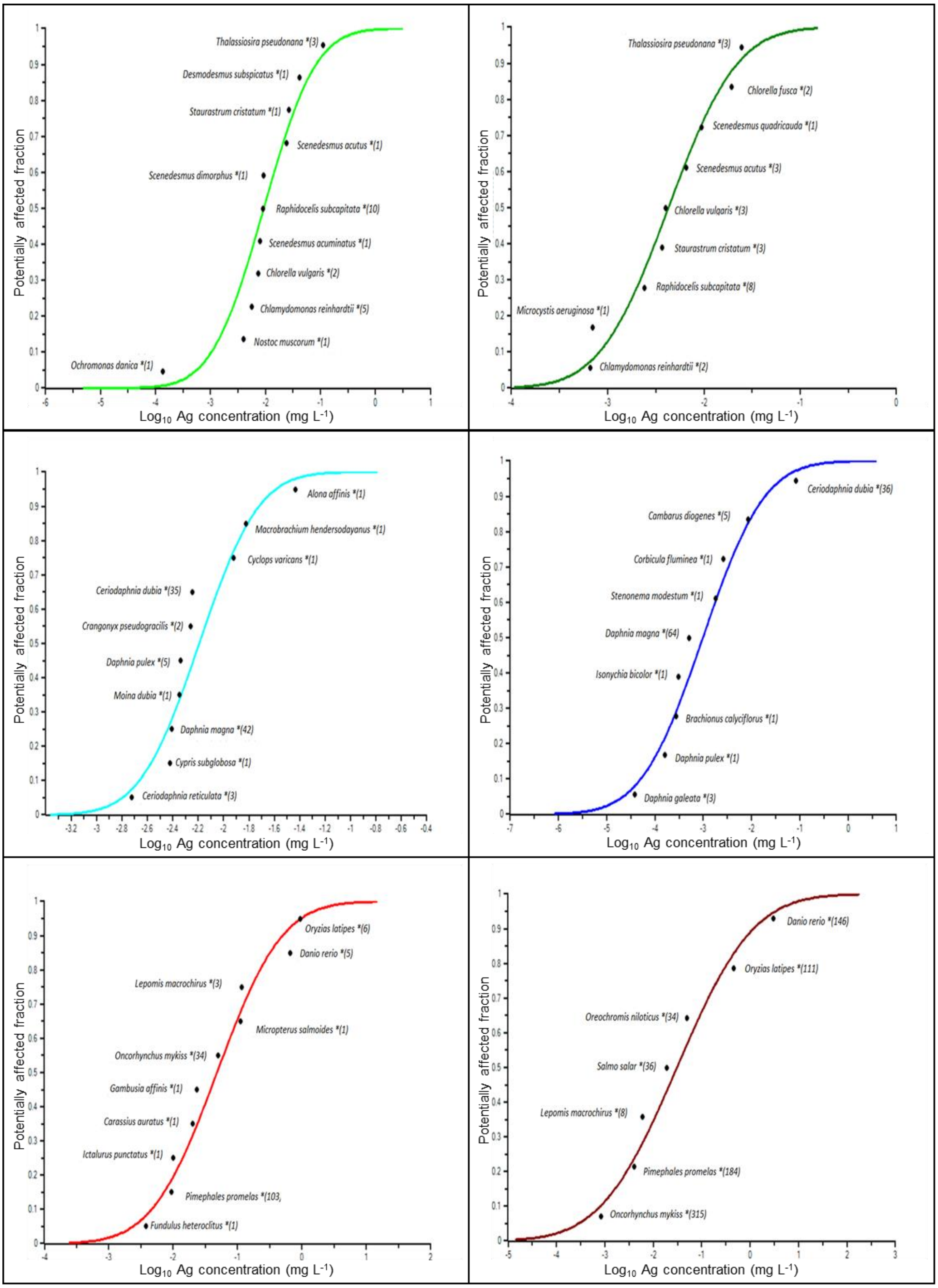
WEE, S. Y.; ARIS, A.Z.; YOSOFF, F.M.; PRAVEENA, S.M. Occurrence and risk assessment of multiclass endocrine disrupting compounds in an urban tropical river and a proposed risk management and monitoring framework. **Science of the Total Environment**, v. 671, p. 431–442, 2019.

WISE URANIUM PROJECT. **Chronology of major tailings dam failure**. Available at: <https://www.wise-uranium.org/mdaf.html>. Access in: 24 Jan. 2023.

YOUNG, G.; CHEN, Y.; YANG, M. Concentrations, distribution, and risk assessment of heavy metals in the iron tailings of Yeshan National Mine Park in Nanjing, China. **Chemosphere**, v. 271, p. 129546, 2021.

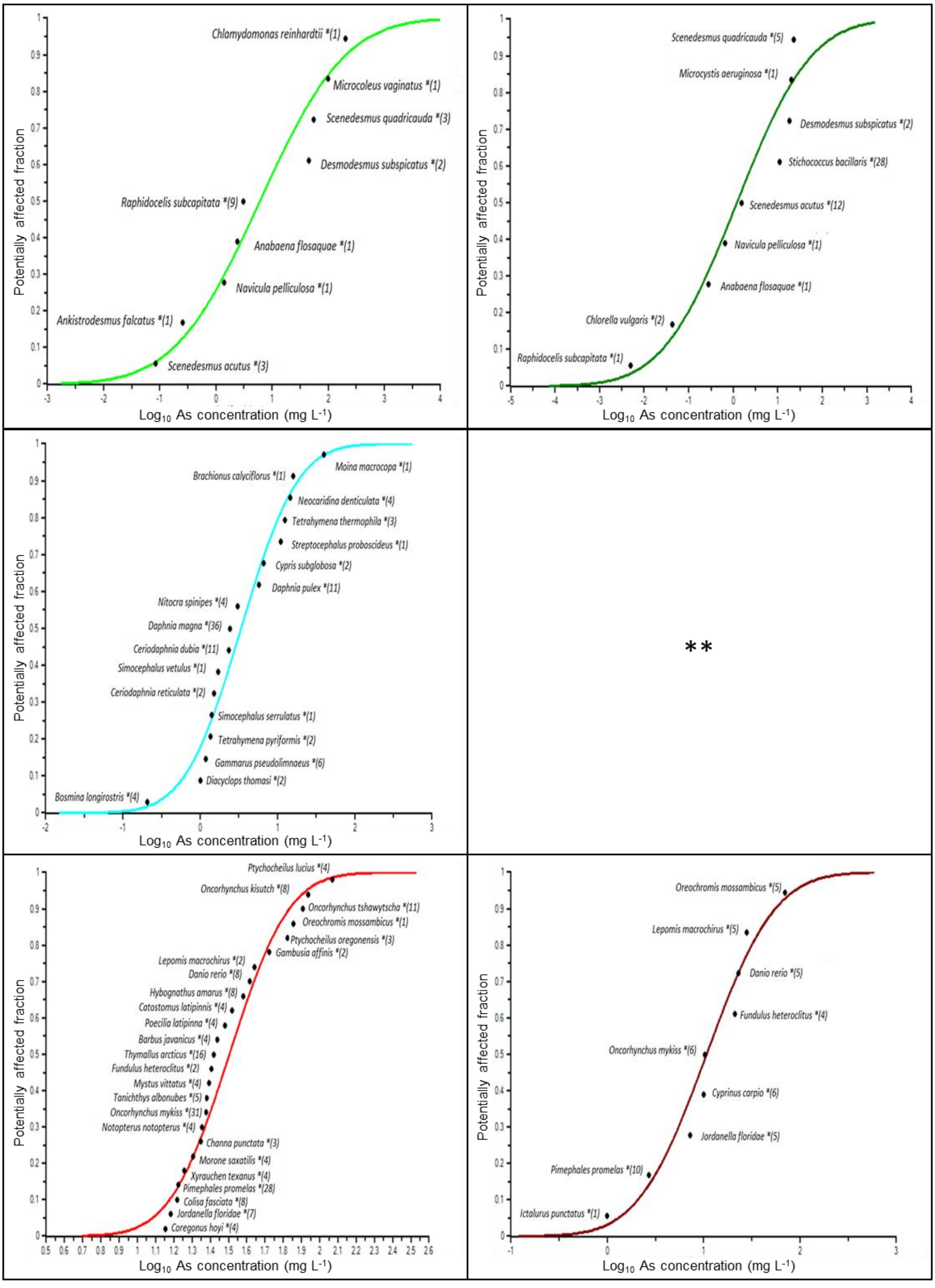
ZHANG, X.; WANG, Q.; LIU, Y.; WU, J.; YU, M. Application of multivariate statistical techniques in the assessment of water quality in the Southwest New Territories and Kowloon, Hong Kong. **Environmental Monitoring and Assessment**, v. 173, p. 17–27, 2011.

APPENDIX A – SSD curves obtained for acute (left) and chronic (right) toxicity of Ag: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



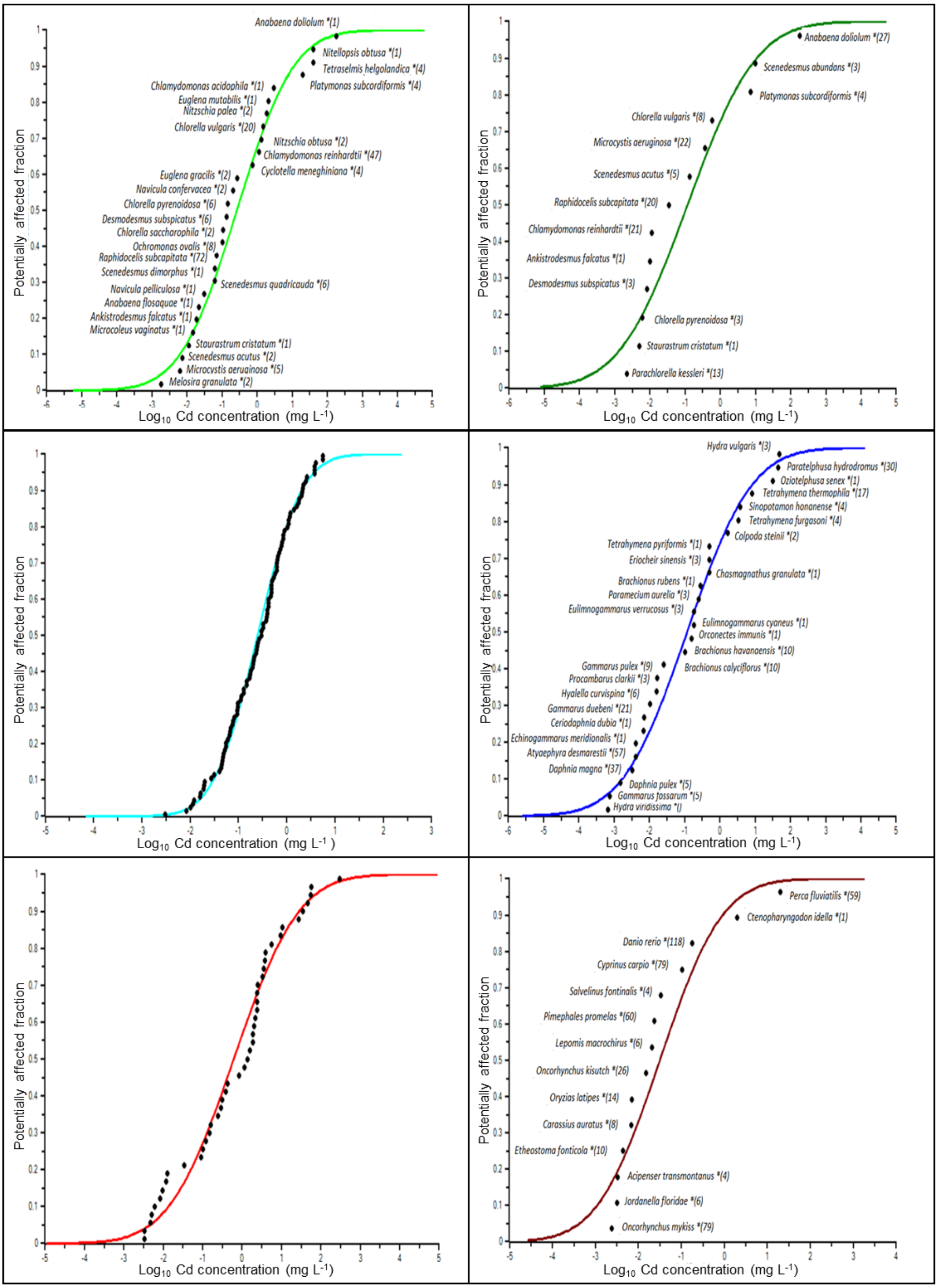
*(X) Number of observations used to plot the SSD curve for each species.

APPENDIX B – SSD curves obtained for acute (left) and chronic (right) toxicity of As: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



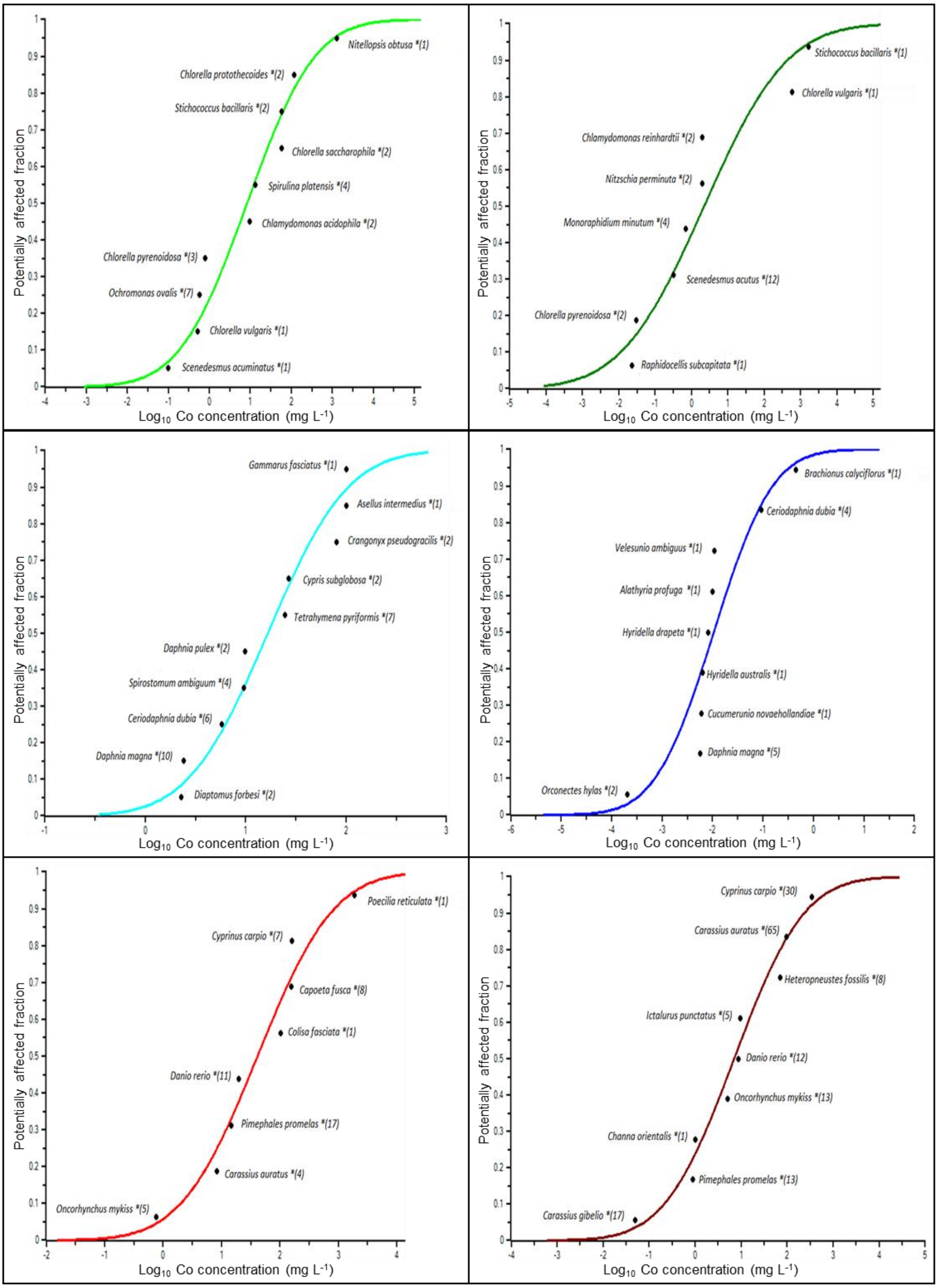
*(X) Number of observations used to plot the SSD curve for each species.
** Curve not obtained due to insufficient number of species.

APPENDIX C – SSD curves obtained for acute (left) and chronic (right) toxicity of Cd: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



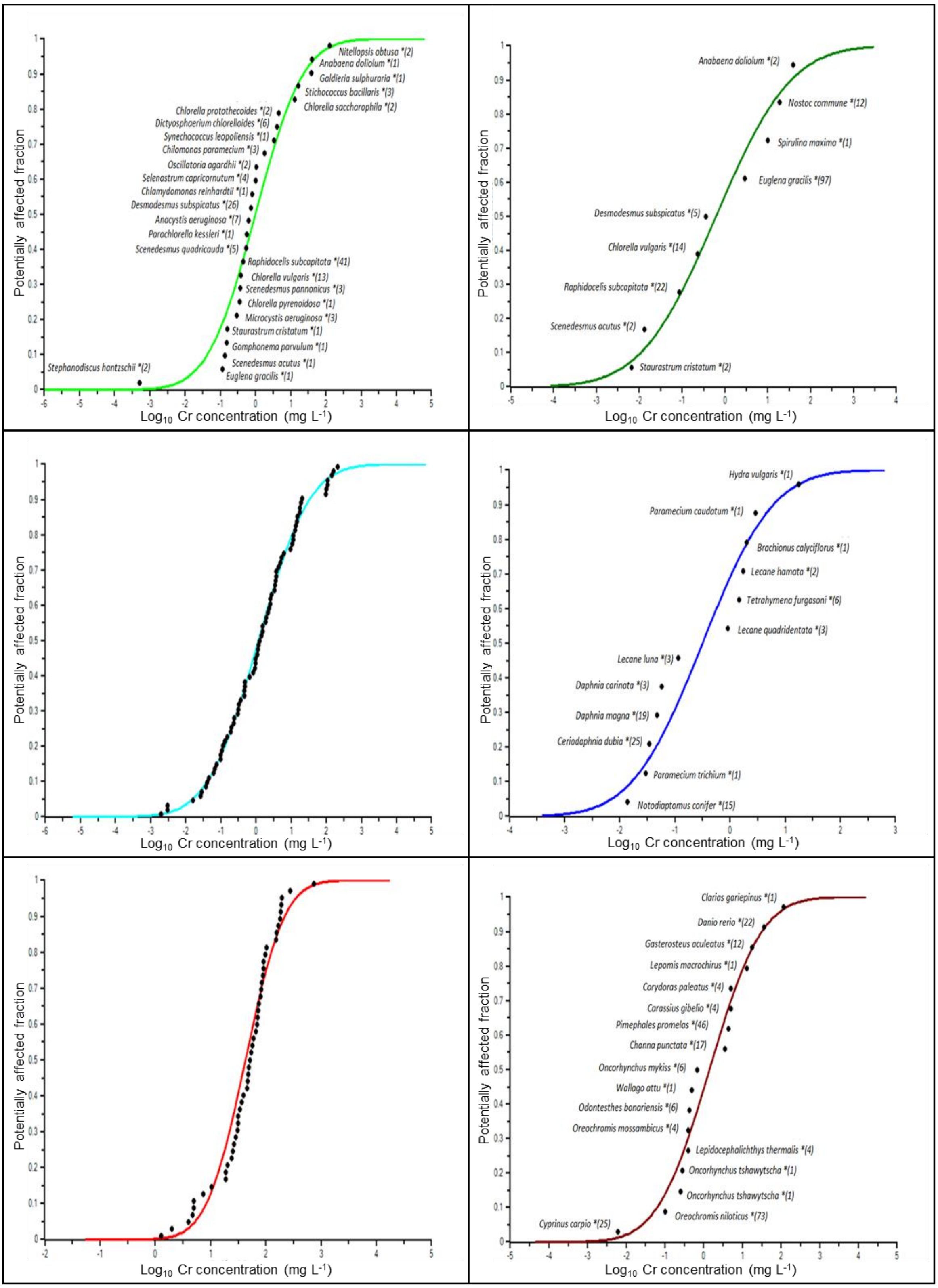
*(X) Number of observations used to plot the SSD curve for each species.

APPENDIX D – SSD curves obtained for acute (left) and chronic (right) toxicity of Co: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



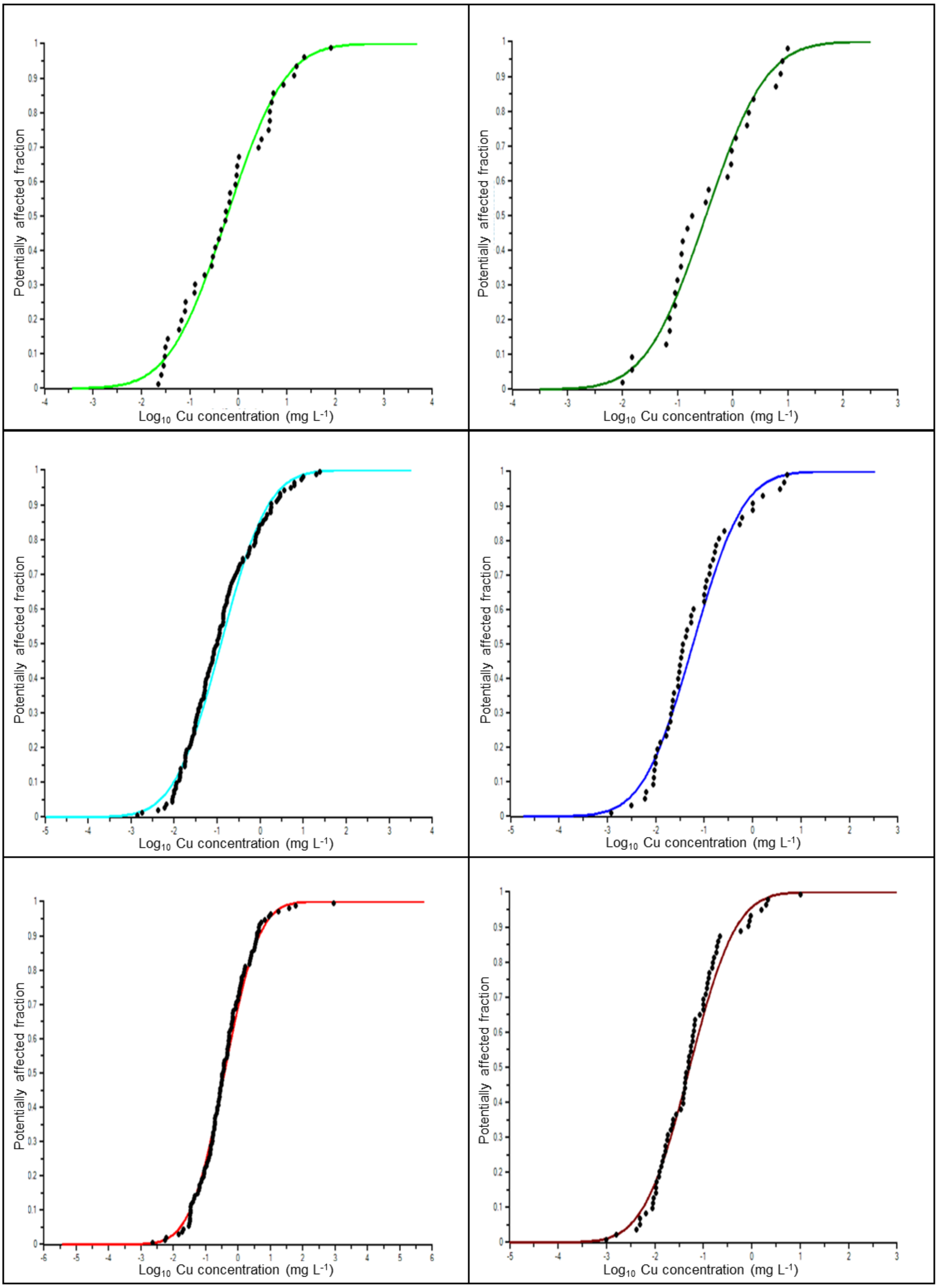
*(X) Number of observations used to plot the SSD curve for each species.

APPENDIX E – SSD curves obtained for acute (left) and chronic (right) toxicity of Cr: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



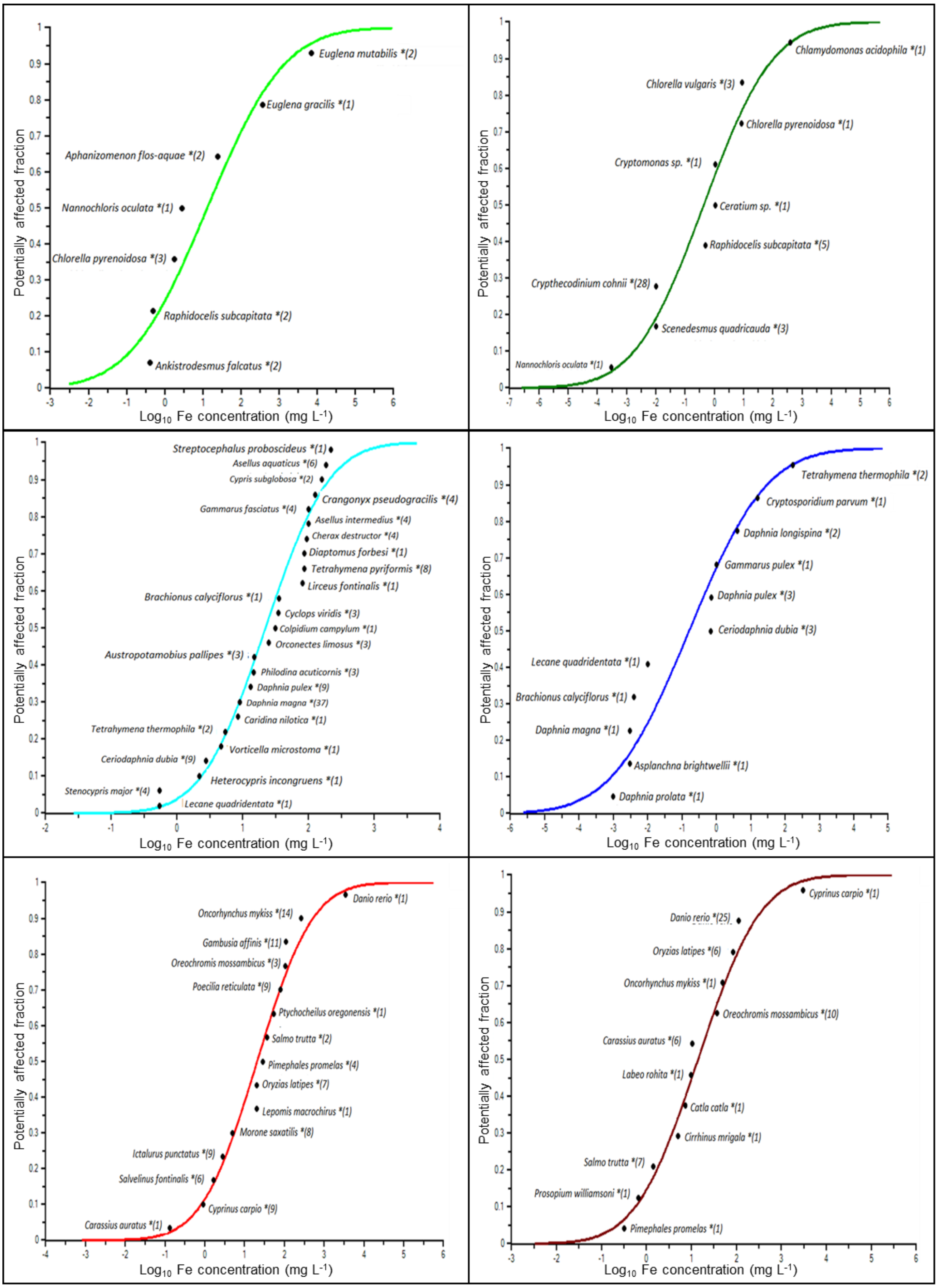
*(X) Number of observations used to plot the SSD curve for each species.

APPENDIX F – SSD curves obtained for acute (left) and chronic (right) toxicity of Cu: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



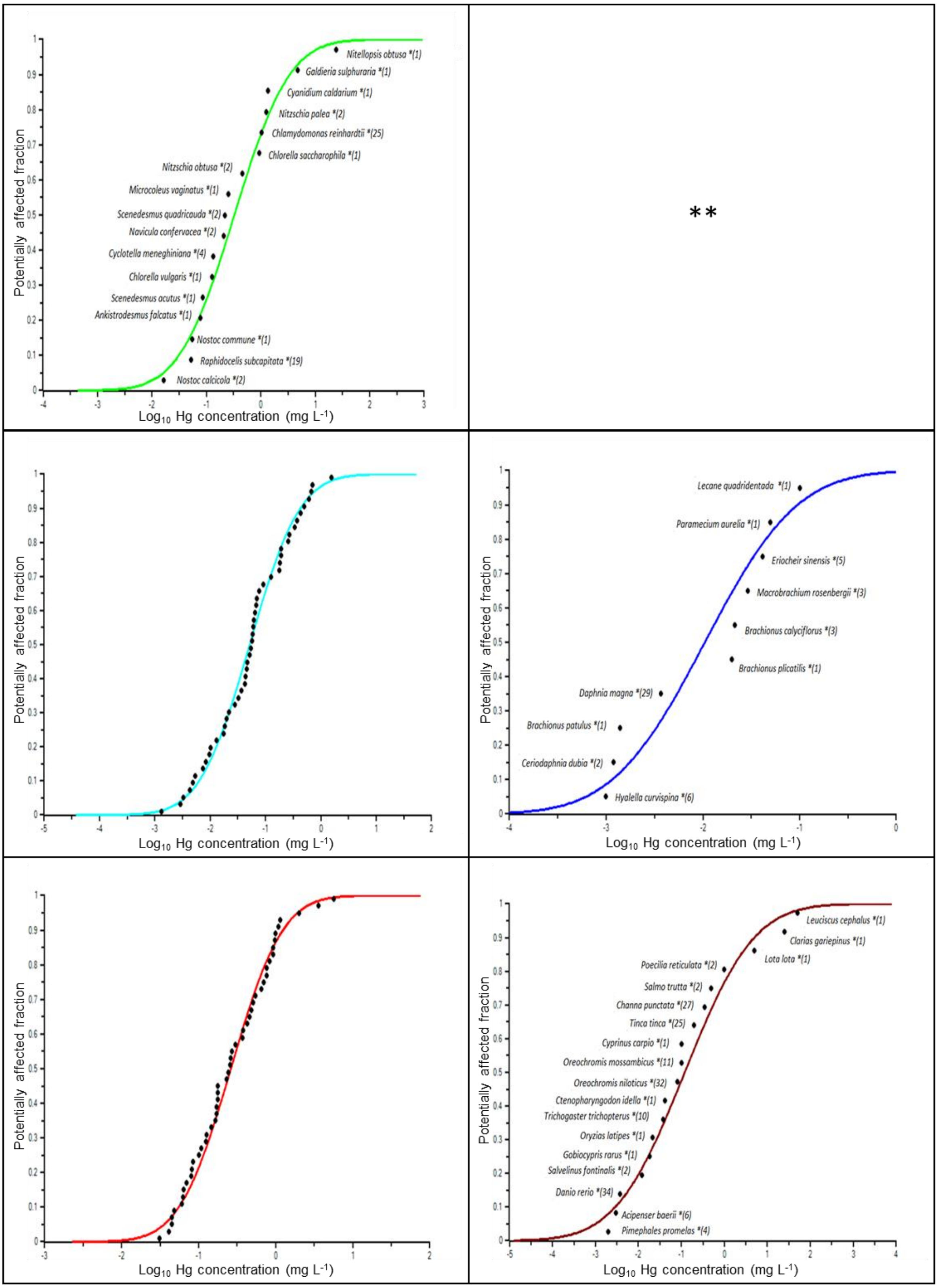
*(X) Number of observations used to plot the SSD curve for each species.

APPENDIX G – SSD curves obtained for acute (left) and chronic (right) toxicity of Fe: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



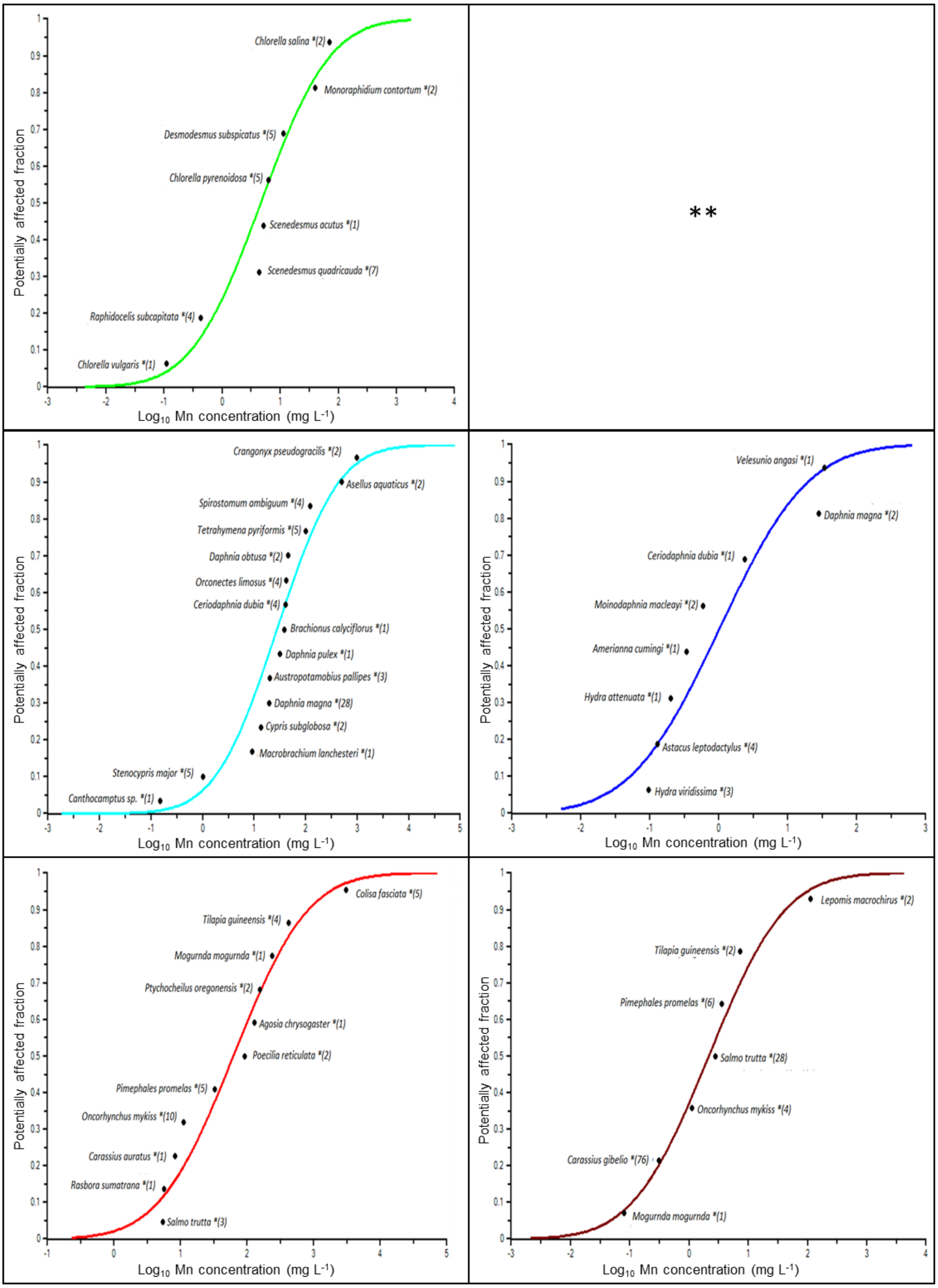
*(X) Number of observations used to plot the SSD curve for each species.

APPENDIX H – SSD curves obtained for acute (left) and chronic (right) toxicity of Hg: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



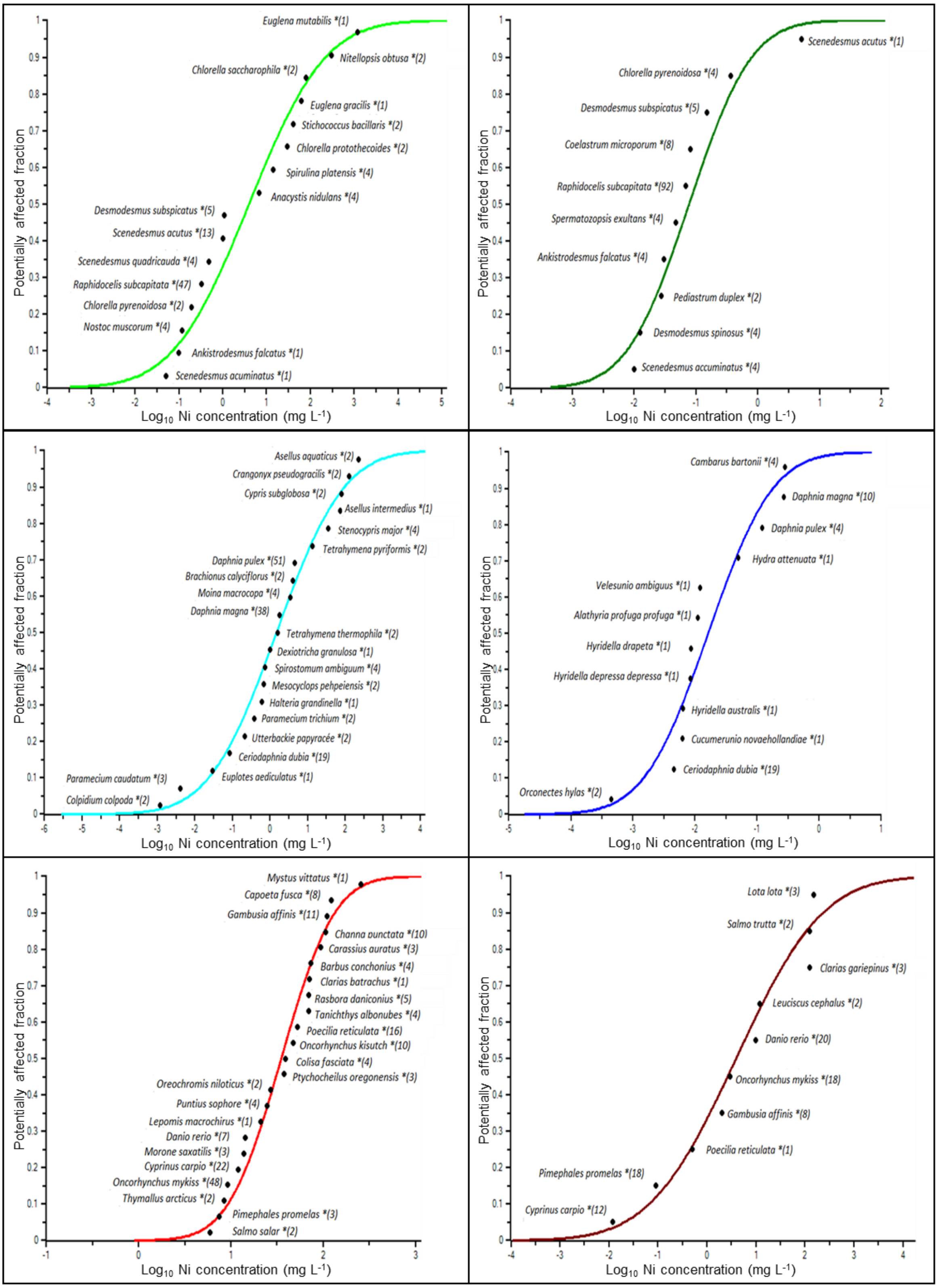
*(X) Number of observations used to plot the SSD curve for each species.
** Curve not obtained due to insufficient number of species.

APPENDIX I – SSD curves obtained for acute (left) and chronic (right) toxicity of Mn: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



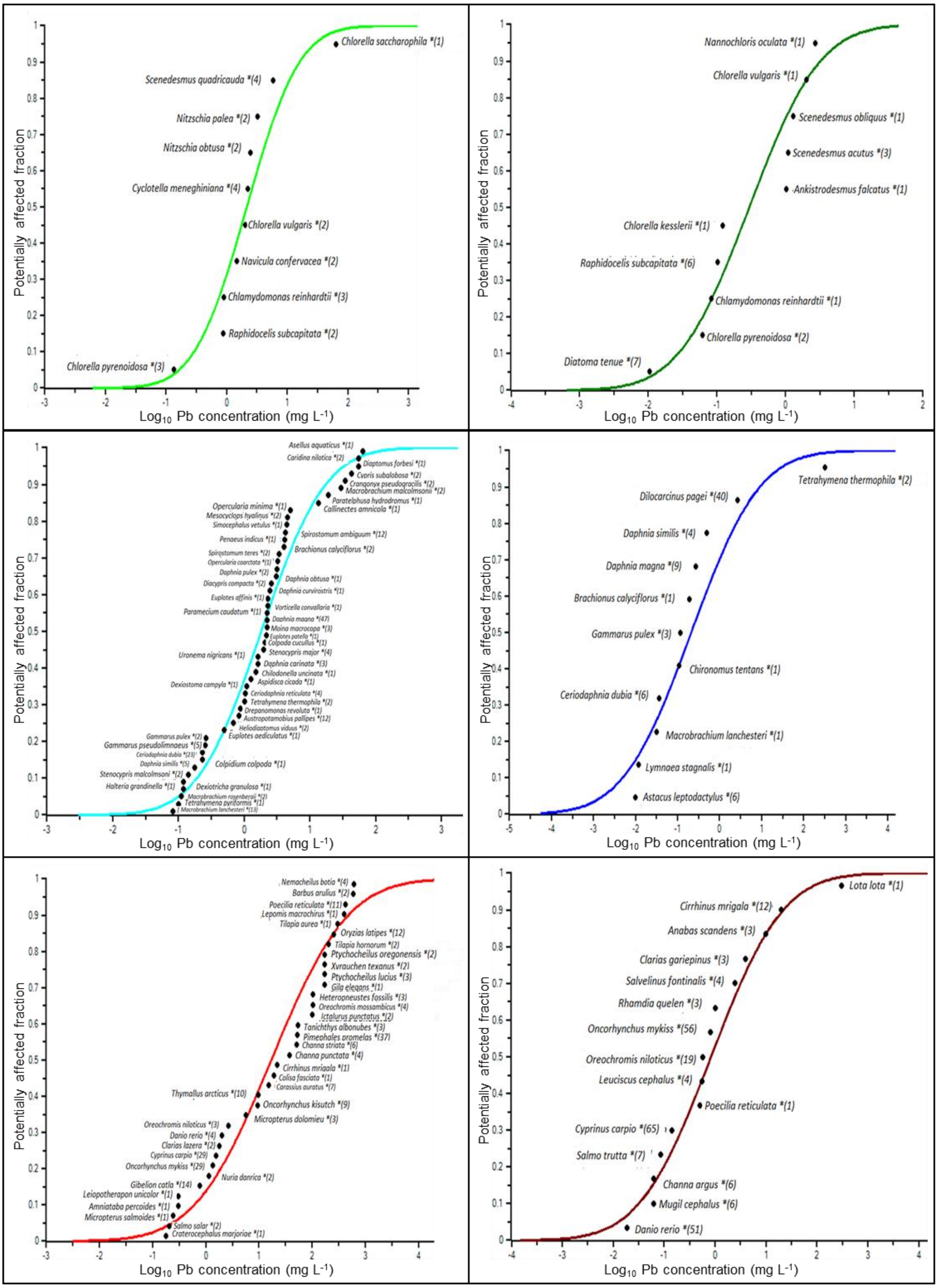
*(X) Number of observations used to plot the SSD curve for each species.
** Curve not obtained due to insufficient number of species.

APPENDIX J – SSD curves obtained for acute (left) and chronic (right) toxicity of Ni: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



*(X) Number of observations used to plot the SSD curve for each species.

APPENDIX K – SSD curves obtained for acute (left) and chronic (right) toxicity of Pb: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



*(X) Number of observations used to plot the SSD curve for each species.

APPENDIX L – SSD curves obtained for acute (left) and chronic (right) toxicity of U: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)

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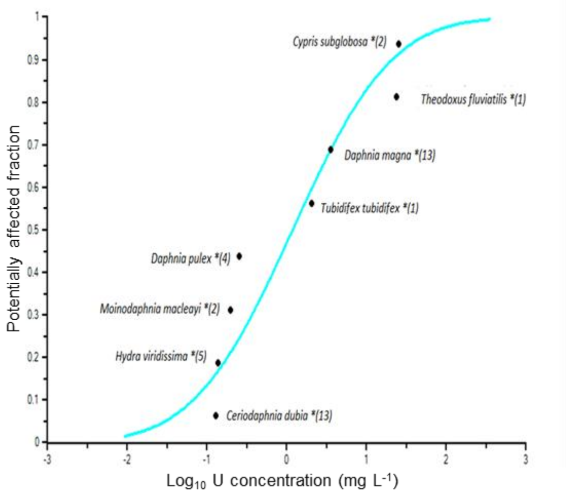

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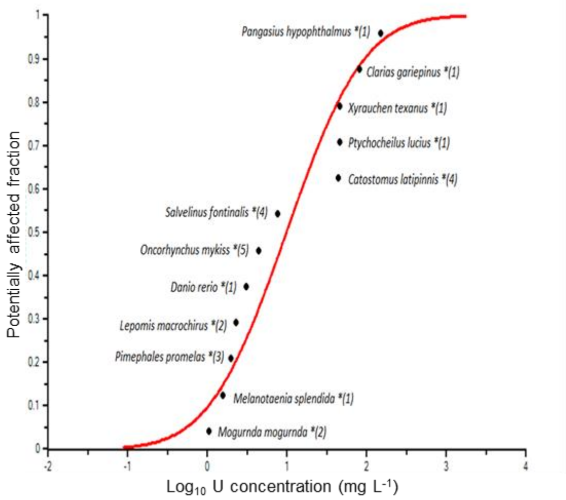
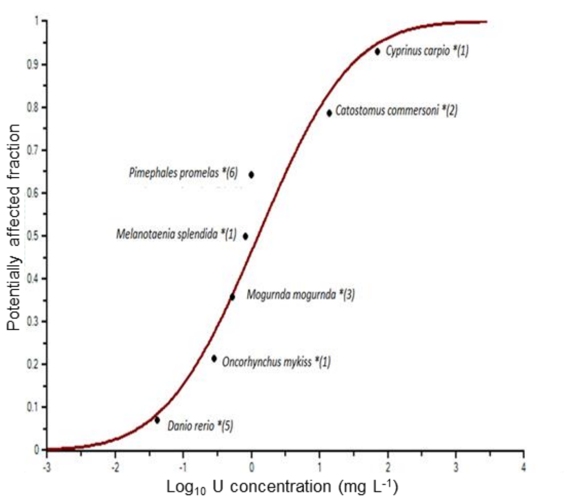
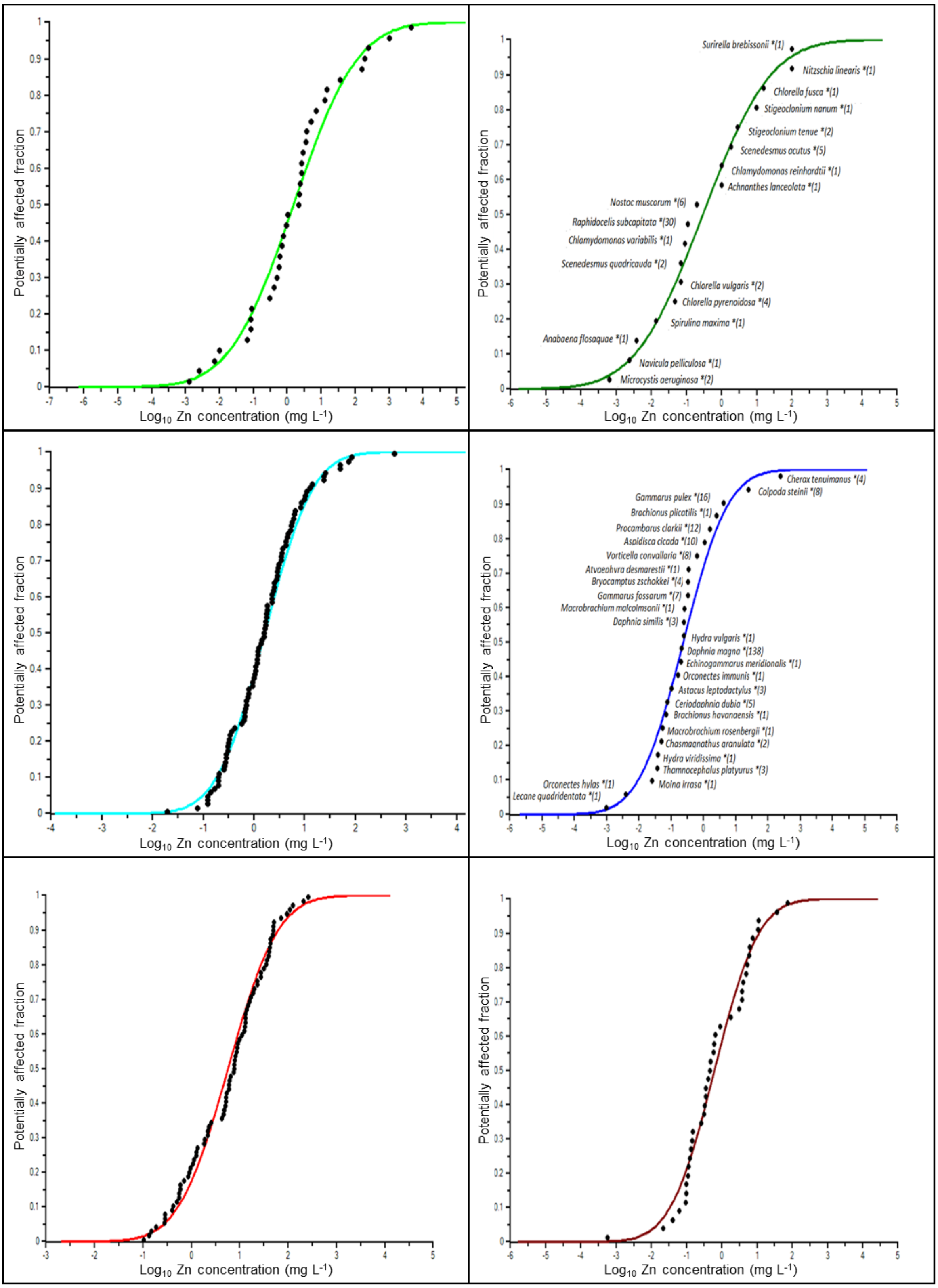




*(X) Number of observations used to plot the SSD curve for each species.
** Curve not obtained due to insufficient number of species.

APPENDIX M – SSD curves obtained for acute (left) and chronic (right) toxicity of Zn: algae/producer (green), invertebrates/primary consumers (blue) and fishes/secondary consumers (red)



*(X) Number of observations used to plot the SSD curve for each species.