UNIVERSIDADE FEDERAL DE SÃO CARLOS – UFSCar
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

DIEGO FERREIRA GOMES

TOXICIDADE DE METAIS: UMA AVALIAÇÃO EM NÍVEIS MOLECULAR, INDIVIDUAL E RISCOS PARA OS ECOSSISTEMAS AQUÁTICOS DA AMAZÔNIA

METALS TOXICITY: AN ASSESSMENT AT MOLECULAR, INDIVIDUAL, LEVELS AND RISKS TO AMAZON AQUATIC ECOSYSTEMS

São Carlos - SP
2024

DIEGO FERREIRA GOMES

METALS TOXICITY: AN ASSESSMENT AT MOLECULAR, INDIVIDUAL LEVELS AND RISKS TO AMAZON AQUATIC ECOSYSTEMS

Tese apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais, Centro de Ciências Biológicas e da Saúde da Universidade Federal de São Carlos – UFSCar, como um dos requisitos para a obtenção do título de Doutor em Ciências, área de concentração em Ecologia e Recursos Naturais.

Orientadora: Profª. Dra. Odete Rocha
Co-orientadora: Profª. Dra. Raquel Aparecida Moreira

São Carlos - SP
2024

UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ecologia e Recursos Naturais

Folha de Aprovação

Defesa de Tese de Doutorado do candidato Diego Ferreira Gomes, realizada em 08/03/2024.

Comissão Julgadora:

Profa. Dra. Odete Rocha (UFSCar)

Profa. Dra. Maria da Graça Gama Melão (UFSCar)

Profa. Dra. Cínthia Bruno de Abreu (UFSCar)

Profa. Dra. Eny Maria Vieira (USP)

Prof. Dr. Thandy Júnio da Silva Pinto (UNICAMP)

O Relatório de Defesa assinado pelos membros da Comissão Julgadora encontra-se arquivado junto ao Programa de Pós-Graduação em Ecologia e Recursos Naturais.

“De tudo ficaram três coisas...
A certeza de que estamos começando...
A certeza de que é preciso continuar...
A certeza de que podemos ser interrompidos antes de terminar...
Façamos da interrupção um caminho novo...
Da queda, um passo de dança...
Do medo, uma escada...
Do sonho, uma ponte...
Da procura, um encontro!”

Fernando Sabino

Agradecimentos

Preciso, inevitavelmente, expressar minha gratidão primeiramente à minha mãe, Ivaneide, ao meu pai, Gilberto, à minha irmã, Michele, e à minha sobrinha, Alice, pessoas cuja existência guia meu caminho. A todos vocês, todo amor que posso sentir e transmitir. Muito obrigado.

Para minha orientadora, Profª. Drª. Odete Rocha, por todo o conhecimento compartilhado. Sem sua visão de mundo, insistência inabalável e resistência nos momentos difíceis para a ciência, talvez hoje eu não estaria na fase final deste doutorado. A você, meus mais sinceros agradecimentos.

Gostaria também de agradecer à minha coorientadora, Profª Dra. Raquel Aparecida Moreira, pois sua gentileza, persistência e amizade mudaram minha vida, fazendo-me redefinir o significado de orientação. Do fundo do meu coração, o meu muito obrigado.

Agradeço à minha companheira Hevelyn, que é a base da minha vida e me lembra todos os dias o que é ser feliz ao lado de alguém. A você minha querida, meu eterno amor.

À equipe do laboratório, em especial à mestranda Larissa, que chegou e trouxe muitas novas cores e energia ao ambiente, e cuja amizade levarei para o resto da vida. Agradeço também aos técnicos Luizinho e Carol pelo apoio ao longo deste estudo.

À Universidade pública, gratuita, aberta e acessível à todos, em especial à Universidade Federal de São Carlos - UFSCar, pela oportunidade de cursar doutorado. Aos departamentos de Ecologia e Biologia Evolutiva (DEBE) e Ciências Fisiológicas (DCF), minha gratidão pelo apoio nesta pesquisa.

Ao Laboratório de Zoofisiologia e Bioquímica Comparada (LZBC) em nome da Profa. Marisa Narcizo Fernandes, que abriu as portas de seu laboratório com um sorriso radiante, muita alegria e ensinamentos. Agradeço também à equipe do laboratório, Maíra e Michelly, pelo apoio durante as análises.

Aos amigos que, ao longo dessa jornada de 4 anos, entraram na minha vida e fizeram dela um lugar melhor, a vocês: Karime Paina, Alef Jonathan, Julia da Silva Pereira, Thandy Junior Pinto, Julia Gomes do Vale, meu sincero agradecimento.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pelo apoio financeiro.

Summary

Estrutura da Tese

Esta tese foi construída e estruturada em quatro capítulos, na qual cada capítulo foi apresentado como artigos científicos, possuindo Resumo, Introdução, Materiais e Métodos, Resultados, Discussão, Conclusões e Referências Bibliográficas de acordo com as normas das revistas científicas nas quais foram publicados ou submetidos.

Capítulo 1- Artigo intitulado "Ecological risk assessment for metals in sediment and waters from the Brazilian Amazon region" – Publicado na revista Chemosphere (A1/FI- 8.8). **DOI:** https://doi.org/10.1016/j.chemosphere.2023.140413**.** Neste trabalho, levantou-se as concentrações de metais para sedimento e água em diferentes regiões da Amazônia legal em estudos que foram publicados nos últimos anos, e com base nessas informações conduziu-se uma avaliação de risco ecológico (ARE) e calculou-se o fator de contaminação dessas regiões.

Capítulo 2 – Artigo intitulado "On a new species of ostracod from the Brazilian Amazon and its potential for experimental studies in laboratory culture" – Submetido na revista Limnologica (A3/FI-1.7). Neste estudo, apresentamos a descrição taxonômica e o ciclo de vida completo da espécie de Ostracoda utilizada como organismo-teste nesta tese.

Capítulo 3 – Artigo intitulado "Toxicity of isolated and mixed metals to a native Amazonian ostracod and ecological risk assessment" – Submetido na revista Environmental Toxicology and Chemistry (A1/FI-4.1)**.** Neste capítulo, avaliamos a toxicidade aguda isolada em mistura para os sais metálicos cobre, cádmio, zinco e mercúrio e com base nessas respostas conduzimos uma avaliação de risco ecológico (ARE).

Capítulo 4 – Artigo intitulado "Integrated Response of Biomarkers of stress and oxidative damage in sublethal exposures to different metals for the ostracod *Strandesia rondoniensis*" – À ser submetido na revista Chemosphere (A1/FI- 8.8). Neste estudo, avaliamos efeitos subletais dos sais metálicos Cobre, Zinco, Cadmio e mercúrio, utilizando biomarcadores como ferramenta de avaliação de estresse e dano oxidativo.

Abstract

The continuous entry of metals into aquatic ecosystems is a global reality, exacerbated in developing countries. In Brazil, especially in the North region, where most of the Legal Amazonis located, multiple pollution sources have multiplied for decades, posing risks to the aquaticbiota. In this perspective, ecotoxicological studies, including the use of biomarkers, and ecological risk assessment protocols, can provide relevant information about the environmental conditions of aquatic ecosystems in this biome. Thus, the main objective of this study was todescribe a new species of ostracode and assess its acute sensitivity to metallic salts ($CuSO_4$, $CdCl_2$, $HgCl_2$, $ZnCl_2$), both individually and in combination, as well as to evaluate if environmentally relevant concentrations induce oxidative stress. Finally, an ecological risk assessment protocol was conducted to examine the risks associated with exposure to these metals. Individuals of the species *Strandesia rondoniensis* used in this study were collected in the Natural Park of Porto Velho, in the Amazon region (state of Rondônia, Brazil), acclimatized, and cultured in laboratory. The growth and reproduction rates of eleven adult individualsof *S. rondoniensis* were analyzed. The ecotoxicological evaluation involved the simultaneous exposure to each metal individually and in a mixture, through a factorial design for toxicity with 25 different combinations over 48 hours. For the ecological risk assessment, concentrations of metals measured in the waters of various aquatic environments in the Amazon basin were considered based on the risk quotient values. Furthermore, investigations into the responses of biomarkers (Reduced Glutathione – GSH, Lipoperoxides – LPO, Metallothionein – MT, Superoxide Dismutase – SOD, and DNA Strandbreak) were conducted for all metals. The results of taxonomic identification of the species showed a high morphological resemblance to *Neostrandesia striata* and *Bradleytriebella lineata*, even though the new species belongs to *Strandesia* genus, indicating evolutionary convergence. Life cycle analysis showed that individuals of *S. rondoniensis* exhibit rapid growth and high reproductive rates, favoring their use in laboratory studies. The ecotoxicological results indicated that the toxicity gradient of metals was Cd > Hg > Cu > Zn. Toxicity in the mixture showed that the combination of Cu-Cd and Cu-Zn fit the model better (CA), indicating primarily synergism when copper predominated in the mixture. Meanwhile, the Cu-Hg interaction fit the model better (IA), again indicating synergism when copper was ata higher concentration. The survey conducted in this study shows that, although metal concentrations in the water were generally low, these values were well above the limits established by current national legislation in many areas, showing higher concentrations for themetals Co, Pb, Cr, Cu, and Ni. Concentrations of Mn, Cu, Ba, Pb, Co, Ni, Cr, Zn, Cd, and As were particularly high in sediment for various evaluated environments. Ecological risk assessment for the water compartment revealed that 56% of the studied areas presented a highrisk (RQ>1) for aquatic biota. In sediment, 66% of the locations showed high risk and 40% medium risk (RQ = 0.1-1). The contamination factor indicated that 49% of the sampling points showed high contamination, and only 24% showed low contamination. Furthermore, sublethalexposures showed that the antioxidant and detoxification system (SOD, GSH and MT) were activated by exposure to metals; however, not enough to contain oxidative damage; thus, increasing LPO levels, resulting in DNA breakage. Therefore, the continuous contamination of aquatic ecosystems inthe Amazon region represents a concerning threat to aquatic biota, as the concentrations foundin our study are considerably above tolerance limits for many species, especially for the ostracode *S. rondoniensis* used in this study.

Keywords: Amazon, Ecological Risk Assessment, Aquatic Ecotoxicology, Native Species, Metals

Resumo

A entrada contínua de metais nos ecossistemas aquáticos é uma realidade global, agravada nos países em desenvolvimento. No Brasil, especialmente na região Norte, onde está localizada a maior parte da Amazônia Legal, múltiplas fontes de poluição se multiplicaram durante décadas, trazendo riscos à biota aquática. Nessa perspectiva, estudos ecotoxicológicos, incluindo o uso de biomarcadores, e protocolos de avaliação de riscos ecológicos, podem fornecer informações relevantes sobre as condições ambientais dos ecossistemas aquáticos deste bioma. Assim, o objetivo principal deste estudo foi descrever uma nova espécie de ostracode e avaliar sua sensibilidade aguda a sais metálicos ($CuSO_4$, $CdCl_2$, $HgCl_2$, $ZnCl_2$), tanto individualmente quanto em combinação, bem como avaliar se concentrações ambientalmente relevantes induzem estresse oxidativo. Finalmente, foi realizado um protocolo de avaliação de risco ecológico para examinar os riscos associados à exposição a estes metais. Indivíduos da espécie *Strandesia rondoniensis* utilizados neste estudo foram coletados no Parque Natural de Porto Velho, na região Amazônica (estado de Rondônia, Brasil), aclimatados e cultivados em laboratório. Foram analisadas as taxas de crescimento e reprodução de onze indivíduos adultos de *S. rondoniensis*. A avaliação ecotoxicológica envolveu a exposição simultânea a cada metal individualmente e em mistura, através de um planejamento fatorial para toxicidade com 25 combinações diferentes ao longo de 48 horas. Para a avaliação do risco ecológico foram consideradas concentrações de metais medidas nas águas de diversos ambientes aquáticos da bacia amazônica com base nos valores do quociente de risco. Além disso, investigações sobre as respostas de biomarcadores (Glutationa Reduzida – GSH, Lipoperóxidos – LPO, Metalotioneína – MT, Superóxido Dismutase – SOD e DNA Strandbreak) foram realizadas para todos os metais. Os resultados da identificação taxonômica das espécies mostraram alta semelhança morfológica com *Neostrandesia striata* e *Bradleytriebella lineata*, embora a nova espécie pertença ao gênero *Strandesia*, indicando convergência evolutiva. A análise do ciclo de vida mostrou que indivíduos de *S. rondoniensis* apresentam rápido crescimento e altas taxas reprodutivas, favorecendo sua utilização em estudos laboratoriais. Os resultados ecotoxicológicos indicaram que o gradiente de toxicidade dos metais foi Cd > Hg > Cu > Zn. A toxicidade na mistura mostrou que a combinação de Cu-Cd e Cu-Zn se ajustou melhor ao modelo (CA), indicando principalmente sinergismo quando o cobre predominou na mistura. Enquanto isso, a interação Cu-Hg ajustou-se melhor ao modelo (IA), novamente indicando sinergismo quando o cobre estava em maior concentração. O levantamento realizado neste estudo mostra que, embora as concentrações de metais na água fossem geralmente baixas, esses valores estavam bem acima dos limites estabelecidos pela legislação nacional vigente em muitas áreas, mostrando concentrações mais elevadas para os metais Co, Pb, Cr, Cu e Não. As concentrações de Mn, Cu, Ba, Pb, Co, Ni, Cr, Zn, Cd e As foram particularmente elevadas nos sedimentos para vários ambientes avaliados. A avaliação do risco ecológico para o compartimento hídrico revelou que 56% das áreas estudadas apresentavam alto risco (RQ>1) para a biota aquática. Nos sedimentos, 66% dos locais apresentaram risco alto e 40% risco médio (RQ = 0,1-1). O fator de contaminação indicou que 49% dos pontos amostrais apresentaram alta contaminação e apenas 24% apresentaram baixa contaminação. Além disso, as exposições subletais mostraram que o sistema antioxidante e de desintoxicação (SOD, GSH e MT) foi ativado pela exposição a metais; porém, não o suficiente para conter o dano oxidativo; assim, aumentando os níveis de LPO, resultando na quebra do DNA. Portanto, a contaminação contínua dos ecossistemas aquáticos na região Amazônica representa uma ameaça preocupante à biota aquática, uma vez que as concentrações encontradas em nosso estudo estão consideravelmente acima dos limites de tolerância para muitas espécies, especialmente para o ostracode *S. rondoniensis* utilizado neste estudo.

Palavras Chave: Amazônia, Avaliação de Risco Ecológico, Ecotoxicologia aquática, Espécies Nativas, Metais

List of Abbreviations

ERA = Ecological Risk Assessment

RQ = Risk quotient

MEC = Measured environmental concentration

PNEC = Predicted no effect concentration

CF = Contamination factor

DV = Dorsal view

H = Height

iv = Internal view

L = Length

RLV = Right lateral view

VV = Ventral view

W = Width

A1 = Antennula

A2 = Antenna

Ex = Exopodite

Mx = Maxillula

R = Rome organ

T1 = First thoracic limb

y3 = Antenna terminal segment aesthethasc

Y = Antenna aesthethasc

ya = Antennula aesthethasc

SSDs = Species sensitivity distributions

ROS = Reactive oxygen species

SOD = Superoxide dismutase

CAT = Catalase

GPx = Glutathione peroxidase

GSH = Glutathione

MT = Metallothionein

1. Introdução Geral

Os ecossistemas aquáticos são altamente importantes para todos os tipos de vida no planeta Terra, porém, nas últimas décadas, diversas ações humanas causaram sérios danos a estes ecossistemas (Chawla, Singh, Haritash, 2023). A introdução de poluentes está geralmente associada a setores econômicos, como agricultura, mineração e diversas atividades industriais, sendo reconhecidas como os principais introdutores de vários poluentes em corpos de água (Zhou et al., 2020).

Dentre os múltiplos poluentes presentes nos ecossistemas aquáticos, os metais podem representar uma ameaça à saúde desses ambientes, acima de seus valores basais (Viana et al., 2021; Jeong et al., 2023). Os metais, podem ser essenciais, desempenhandopapel importante em diversas funções biológicas, sendo responsáveis pela manutenção de diversos processos metabólicos (Andrade et al., 2010), ou não essenciais, não possuindofunção biológica conhecida para os organismos (Koller e Saleh, 2018). A contínua entradade metais (essenciais ou não) nos ecossistemas aquáticos, ao longo do tempo, aumenta as concentrações desses elementos no ambiente (Khan et al., 2011; Ali et al., 2019), principalmente devido sua alta persistência no ambiente, estabilidade ambiental, e potencial de bioacumulação na biota (Ustaoglu e Islam, 2020; Zhang et al., 2019). O problema dessa situação é que, em altas concentrações, esses contaminantes tornam-se tóxicos para a biota aquática (Rosabal et al., 2015; Ali et al., 2019), bem como, a longo prazo, representa um risco à saúde humana (Gomes et al., 2020; Weber et al., 2020).

Embora a contaminação por metais seja um problema mundial (Viana et al., 2021; Liu et al., 2020), nos países em desenvolvimento esta situação pode ser ainda mais alarmante (Mohiuddin et al., 2011). Inserido neste contexto, o Brasil possui dimensões continentais e, por isso, enfrenta inúmeros desafios na preservação dos recursos hídricos. Segundo dados apresentados pela Agência Nacional de Águas - ANA (Brasil, 2017), 38% da população brasileira não dispõe de tratamento de esgoto, sendo a região Norte do país especialmente afetada, com cerca de 86% dos municípios sem qualquer tipo de sistema de coleta e tratamento de esgoto ou abastecimento de água potável. Esta situação tem suscitado preocupações na comunidade científica, uma vez que a região Norte abrange uma parte significativa da Amazônia Legal.

A Amazônia legal é uma floresta urbanizada, culturalmente diversa e com forte mobilidade intrarregional. A biodiversidade da Amazônia está distribuída em diferentes ambientes representados por floresta de sequeiro, cerrado, floresta de igapó e várzeas, tornando o Brasil um dos países com maior biodiversidade do planeta (Pereira, 2017).

Contudo, o desenvolvimento territorial nos estados que abrangem a Amazônia Legal ocorreu de forma acelerada e desordenada, especialmente entre as décadas de 1960 e 1970, quando o governo militar brasileiro promoveu ativamente a exploração mineral, o agronegócio, a construção de rodovias, usinas hidrelétricas e outros grandes projetos na região amazônica (Rico et al., 2021), resultando em intenso fluxo migratório para a região (Matos, 2005; Barbieri, 2007). Como resultado da situação apresentada acima, existem evidências concretas sobre a entrada contínua de metais neste bioma e os riscos associados a esse cenário (Moulatlet et al., 2023; Gomes et al., 2023).

Nessa perspectiva, dentre as diversas técnicas e métodos utilizados no monitoramento ambiental, a Ecotoxicologia aquática tem ganhado destaque nas últimas décadas (Costa et al., 2008). Esta ciência é definida como o estudo dos efeitos das substâncias, sejam elas naturais ou sintéticas, sobre os organismos vivos, populações ou comunidades, tanto animais como vegetais, terrestres e aquáticos, que constituem a biosfera. Isto inclui a análise da interação dessas substâncias com o ambiente em que vivem os organismos, dentro de um contexto integrado (Gherardi-Goldstein et al., 1990; Bertoletti, 2006).

Os testes ecotoxicológicos são realizados por meio de análises laboratoriais em condições experimentais específicas e controladas. Portanto, os estudos toxicológicos desempenham um papel crucial na estimativa dos riscos associados a um determinado agente químico (isolado ou em mistura), bem como as condições de exposição que podem aumentar ou reduzir esses riscos (James et al., 2000). Por outro lado, a necessidade crescente de detectar e avaliar o impacto da poluição, especialmente em concentrações mais baixas para uma variedade de substâncias complexas, levou ao desenvolvimento de marcadores moleculares para os efeitos biológicos dos contaminantes nos organismos, conhecidos como biomarcadores. Esses biomarcadores são fundamentais para avaliar a qualidade ambiental e compreender misturas complexas de contaminantes (Livingstone, 1993, 2001).

Dentre os diversos grupos utilizados como organismos teste, os ostracodes se destacam por serem formados por pequenos crustáceos bentônicos, que desempenham um papel importante na comunidade aquática. Caracterizam-se por possuírem comprimento corporal que pode variar de 0,3 a 3mm, e seu corpo possui apêndices que são circundados por uma carapaça bivalvulada que os diferencia de outros grupos de crustáceos (Horne et al., 2002). A locomoção desses organismos é realizada através do auxílio de apêndices modificados, que podem ser robustos, como garras e pernas fortes

para caminhar e escalar ou cerdas nadadoras para nadar (Meisch, 2000). Recentemente, os ostracoda ganharam destaque em estudos ecotoxicológicos. Isso porque possuem algumas características como: pequeno tamanho e possibilidade de serem utilizados em testes de pequena escala (Khangarot; Das, 2009). São facilmente cultivados em laboratório (Kesling, 1951; Cohen, 1983; Havel e Hebert, 1989). Essas características têm despertado o interesse na utilização deste grupo para estudos ecotoxicológicos, a saber: estressores ambientais (Anadón; Gliozzi; Mazzini, 2002; Pascual, et al. 2002), metais (Khangarot; Ray, 1987; Bergin, et al. 2006; Dos Santos et al., 2023), lodo de esgoto (Oleszczuk, 2008a; Oleszczuk, 2008b) e efluentes industriais (Khangarot et al., 1983; Sanchez-Bayo, 2006).

1. **General Introduction**

Aquatic ecosystems are highly important for all types of life on planet; however, in recent decades, several human activities have caused serious damage to these ecosystems (Chawla, Singh, Haritash, 2023). The introduction of pollutants is generally associated with economic sectors, such as agriculture, mining, and different industrial activities, being recognized as the main activities responsible for the introduction of various pollutants into water bodies (Zhou et al., 2020).

Among the multiple pollutants present in aquatic ecosystems, metals represent a threat to the health of these environments (Viana et al., 2021; Jeong et al., 2023). Metals, can be essential, playing an important role in various biological functions, being responsible for maintaining different metabolic processes (Andrade et al., 2010), or non-essential, having no biological function described for organisms (Koller and Saleh, 2018).The continuous entry of metals (essential or not) into aquatic ecosystems, over time, increases the concentrations of these elements in the environment (Khan et al., 2011; Ali et al., 2019), due to their high persistence, bioaccumulation potential, and environmental stability (Ustaoglu and Islam, 2020; Zhang et al., 2019). In high concentrations, normally above their basal limits, these metals become toxic to the aquatic biota (Rosabal et al., 2015; Ali et al., 2019), as well as, in the long term, it poses a risk to human health (Gomes et al., 2020; Weber et al., 2020).

Although metal contamination is a problem worldwide (Viana et al., 2021; Liu et al., 2020), in developing countries this situation can be even more alarming (Mohiuddin et al., 2011). In this context, Brazil has continental dimensions, and for this reason, it faces numerous challenges in preserving water resources. According to data presented by

the National Water Agency - ANA (Brazil, 2017), 38% of the Brazilian population does not have sewage treatment, with the northern region of the country being especially affected, with around 86% of municipalities without any type of sewage treatment or supply of drinking water. This situation has raised concerns in the scientific community since the northern region covers a significant part of the Legal Amazon.

The legal Amazon is an urbanized forest, culturally diverse and with strong intra-regional mobility. The biodiversity of the Amazon is distributed in different environments represented by dryland forest, savanna, igapó forest and floodplains, making Brazil one of the countries with the greatest biodiversity on the planet (Pereira, 2017). However, territorial development in the states that cover the legal Amazon occurred in an accelerated and disorderly manner, especially between the 1960s and 1970s, when the Brazilian military government actively promoted mineral exploration, agribusiness, construction of highways, hydroelectric plants and other large projects in the Amazon region (Rico et al., 2021), resulting in an intense migratory flow to the region (Matos, 2005; Barbieri, 2007). In this way, there is concrete evidence about the continuous entry of metals into aquatic ecosystems from Amazon and their associated risks (Moulatlet et al., 2023; Gomes et al., 2023).

From this perspective, among the various techniques and methods used in environmental monitoring, aquatic Ecotoxicology has gained prominence in recent decades (Costa et al., 2008). This science is defined as the study of the effects of substances, whether natural or synthetic, on living organisms, populations or communities, both animals and plants, both terrestrial and aquatic, that make up the biosphere. This includes the analysis of the interaction of these substances with the environment in which the organisms live, within an integrated context (Gherardi-Goldstein et al., 1990; Bertoletti, 2006).

Ecotoxicological tests are carried out through laboratory analyzes under specific and controlled experimental conditions. Therefore, toxicological studies play a crucial role in estimating the risks associated with a given chemical agent (alone or in mixture), as well as the exposure conditions that can increase or reduce these risks (James et al., 2000). On the other hand, the increasing demand for detectingand assessing the impacts of water pollution, especially at lower concentrations ofa variety of complex substances, leds to the development of molecular markers for the biological effects of contaminants on organisms, known as biomarkers. These biomarkers are fundamental for assessing

environmental quality and understanding complex mixtures of contaminants (Livingstone, 1993, 2001).

Among the many groups used as test organisms, ostracods are small and more diverse benthic crustaceans, standing out among the aquatic community. Their body length ranges from 0.3 to 3mmand contain appendages that are surrounded by a bivalved carapace that differentiates them from other groups of crustaceans (Horne et al., 2002). Besides, modified appendages are responsible for their locomotion. These structures can be robust, such as strong claws and legs for walking and climbing, or swimming bristles for swimming (Meisch, 2000). Recently, ostracoda have gained prominence in ecotoxicological studies, mainly due to their small size whose allows to small-scale tests (Khangarot; Das, 2009). Furthermore, they are easily cultivated in the laboratory (Kesling, 1951; Cohen, 1983; Havel and Hebert, 1989). These characteristics have sparked interest in using this group for ecotoxicological studies, namely: environmental stressors (Anadón; Gliozzi; Mazzini, 2002; Pascual, et al. 2002), metals (Khangarot; Ray, 1987; Bergin, et al. 2006; Dos Santos et al., 2023), sewage sludge (Oleszczuk, 2008a; Oleszczuk, 2008b) and industrial effluents (Khangarot et al., 1983; Sanchez-Bayo, 2006).

References

Agência Nacional de Águas - Esgotos, A. (2017). despoluição de bacias hidrográficas. *Agência Nacional de Águas, Secretaria Nacional de*.

Ali, H., Khan, E., Ilahi, I., (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry*, 1–14.

Anadón, P., Gliozzi, E., & Mazzini, I. (2002). Paleoenvironmental reconstruction of marginal marine environments from combined paleoecological and geochemical analyses on ostracods. *The Ostracoda: applications in quaternary research*, *131*, 227-247.

Andrade, M. T., Veador, M. A. R. V., Menezes, M. A. B. C., & ALÍPIO, V. D. C. (2010). Análise da concentração de metais pesados no rio Piracicaba, Minas Gerais. *Encontro Nac Eng Produção*, *1*, 1-15.

Barbieri, A. F., (2007). Towns in the Jungle: Exploring Linkages between Rural-Urban Mobility, Urbanization, and Development in the Amazon Center for Regional Development and Planning (CEDEPLAR), Federal University of Minas Gerais (UFMG), Brazil, and Richard E. Bilsborrow (North).

Bergin, F., Kucuksezgin, F., Uluturhan, E. S. İ. N., Barut, I. F., Meric, E., Avsar, N. İ. Y. A. Z. İ., & Nazik, A. T. İ. K. E. (2006). The response of benthic foraminifera and ostracoda to heavy metal pollution in Gulf of Izmir (Eastern Aegean Sea). *Estuarine, Coastal and Shelf Science*, *66*(3-4), 368-386.

Bertoletti, E., & Zagatto, P. A. (2006). Aplicação dos ensaios ecotoxicológicos e Legislação pertinente. *Ecotoxicologia Aquática Princípios e Aplicações. Rima. São Paulo. São Carlos. 478p*, 347-379.

Chawla, H., Singh, S. K., & Haritash, A. K. (2023). Reversing the damage: ecological restoration of polluted water bodies affected by pollutants due to anthropogenic activities. *Environmental Science and Pollution Research*, 1-17.

Cohen, A. C. (1983). Rearing and postembryonic development of the myodocopid ostracode Skogsbergia lerneri from coral reefs of Belize and the Bahamas. *Journal of Crustacean Biology*, *3*(2), 235-256.

Costa, C. R., Olivi, P., Botta, C. M., & Espindola, E. L. (2008). A toxicidade em ambientes aquáticos: discussão e métodos de avaliação. *Química nova*, *31*, 1820-1830.

dos Santos Lima, J. C., Moreira, R. A., Neto, A. J. G., Freitas, E. C., Daam, M. A., Rocha, O. (2023). Toxicity of Binary Metal Mixtures to the Tropical Ostracod *Strandesia trispinosa*. *Bulletin of Environmental Contamination and Toxicology*, *110* (3), 64.

Gherardi-Goldstein, E., Bertoletti, E., Zagatto, P. A., Araújo, R. A., Ramos, M. L. L. C. (1990). Procedimentos para utilização de testes de toxicidade no controle de efluentes líquidos. *São Paulo: CETESB*.

Gomes, D. F., da Silva Pinto, T. J., Raymundo, L. B., da Fontoura Sperandei, V., Daam, M., Moreira, R. A., & Rocha, O. (2023). Ecological risk assessment for metals in sediment and waters from the Brazilian Amazon region. *Chemosphere*, *345*, 140413.

Gomes, D. F., Moreira, R. A., Sanches, N. A. O., do Vale, C. A., Daam, M. A., Gorni, G. R., & Bastos, W. R. (2020). Dynamics of (total and methyl) mercury in sediment, fish,

and crocodiles in an Amazonian Lake and risk assessment of fish consumption to the local population. *Environmental monitoring and assessment*, *192*, 1-10.

Havel, J. E., & Hebert, P. D. (1989). Apomictic parthenogenesis and genotypic diversity in Cypridopsis vidua (Ostracoda: Cyprididae). *Heredity*, *62*(3), 383-392.

Horne, D. J.; Cohen, A. & Martens, K. (2002). Taxonomy, morphology and biology of quaternary and living Ostracoda. In: Holmes J. A. & Chivas, A. R. eds. The Ostracoda: Applications in quaternary research geophysical monograph. Washington D.C., American Geophysical Union. (Geophysical Monograph). Vol. 131, p. 5-36.

James, R. C., Roberts, S. M., & Williams, P. L. (2000). General principles of toxicology. *Principles of toxicology: environmental and industrial applications*, 1-34.

Jeong, H., Byeon, E., Kim, D. H., Maszczyk, P., Lee, J. S. (2023). Heavy metals and metalloid in aquatic invertebrates: A review of single/mixed forms, combination with other pollutants, and environmental factors. *Marine Pollution Bulletin*, *191*, 114959.

Kesling, R. V. (1949). *The morphology of ostracod molt stages*. University of Illinois at Urbana-Champaign.

Khan T., Muhammad S., Khan B., and Khan H., (2011). Investigating the levels of selected heavy metals in surface water of shah alam river (a tributary of River Kabul, Khyber Pakhtunkhwa), *Journal of Himalayan Earth Sciences*, 44, (2) 71–79.

Khangarot, B. S., & Das, S. (2009). Acute toxicity of metals and reference toxicants to a freshwater ostracod, Cypris subglobosa Sowerby, 1840 and correlation to EC50 values of other test models. *Journal of hazardous materials*, *172*(2-3), 641-649.

Rao, P. S., Durve, V. S., Khangarot, B. S., & Shekhawat, S. S. (1983). Acute toxicity of phenol, pentachlorophenol and sodium pentachlorophenate to a freshwater ostracod Cypris subglobosa (Sowerby). *Acta hydrochimica et hydrobiologica*, *11*(4), 457-465.

Khangarot, B. S., & Ray, P. K. (1987). Response of a freshwater ostracod (Cypris subglobosa Sowerby) exposed to copper at different pH levels. *Acta hydrochimica et hydrobiologica*, *15*(6), 553-558.

Koller, M., Saleh, H. M., (2018). Introductory chapter: Introducing heavy metals. *Heavy metals*, *1*, 3-11.

Lamine, I., Elazzaoui, A., Ben-Haddad, M., Agnaou, M., Moukrim, A., & Ait Alla, A. (2023). Integrated biomarker responses and metal contamination survey in the wedge clam Donax trunculus from the Atlantic coast of Morocco. *Environmental Science and Pollution Research*, *30*(13), 38465-38479.

Liu, P., Hu, W., Tian, K., Huang, B., Zhao, Y., Wang, X., Khim, J. S. (2020). Accumulation and ecological risk of heavy metals in soils along the coastal areas of the Bohai Sea and the Yellow Sea: A comparative study of China and South Korea. *Environment international*, *137*, 105519.

Livingstone, D. R. (1993). Biotechnology and pollution monitoring: use of molecular biomarkers in the aquatic environment. *Journal of Chemical Technology & Biotechnology*, *57*(3), 195-211.

Livingstone, D. R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine pollution bulletin*, *42*(8), 656-666.

Matos, R., (2005). Espacialidades em rede: população, urbanização e migração no Brasil contemporâneo. *Belo Horizonte: C/Arte*.

Matyja, K. (2023). Sublethal effects of binary mixtures of Cu2+ and Cd2+ on Daphnia magna: Standard Dynamic Energy Budget (DEB) model analysis. *Environmental Pollution*, *334*, 122142.

Meisch, C. (2000). Freshwater Ostracoda of western and central Europe. *Sußwasserfauna von Mitteleuropa 8/3*.

Mohiuddin, K. M., Ogawa, Y. Z. H. M., Zakir, H. M., Otomo, K., Shikazono, N. (2011). Heavy metals contamination in water and sediments of an urban river in a developing country. *International journal of environmental science & technology*, *8*, 723-736.

Moulatlet, G. M., Yacelga, N., Rico, A., Mora, A., Hauser-Davis, R. A., Cabrera, M., Capparelli, M. V. (2023). A systematic review on metal contamination due to mining activities in the Amazon basin and associated environmental hazards. *Chemosphere*, 139700.

Oleszczuk, P. (2008). Heterocypris incongruens as a tool to estimate sewage sludge toxicity. *Environmental Toxicology and Chemistry: An International Journal*, *27*(4), 864-872.

Oleszczuk, P. (2008). The toxicity of composts from sewage sludges evaluated by the direct contact tests phytotoxkit and ostracodtoxkit. *Waste management*, *28*(9), 1645-1653.

Pereira, J. O., de Souza, A. Q. L., de Souza, A. D. L., de Castro França, S., de Oliveira, L. A. (2017). Overview on biodiversity, chemistry, and biotechnological potential of microorganisms from the Brazilian Amazon. *Diversity and Benefits of Microorganisms from the Tropics*, 71-103.

Rico, A., de Oliveira, R., de Souza Nunes, G. S., Rizzi, C., Villa, S., López-Heras, I., Waichman, A. V. (2021). Pharmaceuticals and other urban contaminants threaten Amazonian freshwater ecosystems. *Environment International*, *155*, 106702.

Rosabal, M., Pierron, F., Couture, P., Baudrimont, M., Hare, L., Campbell, P. G., 2015. Subcellular partitioning of non-essential trace metals (Ag, As, Cd, Ni, Pb, and Tl) in livers of American (Anguilla rostrata) and European (Anguilla anguilla) yellow eels. *Aquatic Toxicology*, *160*, 128-141.

Sanchez-Bayo, F. (2006). Comparative acute toxicity of organic pollutants and reference values for crustaceans. I. Branchiopoda, Copepoda and Ostracoda. *Environmental Pollution*, *139*(3), 385-420.

Ustaoğlu, F., Islam, M. S. (2020). Potential toxic elements in sediment of some rivers at Giresun, Northeast Turkey: A preliminary assessment for ecotoxicological status and health risk. *Ecological indicators*, *113*, 106237.

Viana, L. F., Kummrow, F., Cardoso, C. A. L., de Lima, N. A., Solórzano, J. C. J., do A. Crispim, B., Florentino, A. C. (2021). High concentrations of metals in the waters from Araguari River lower section (Amazon biome): Relationship with land use and cover, ecotoxicological effects and risks to aquatic biota. *Chemosphere*, 285, 131451.

Weber, A. A., Sales, C. F., de Souza Faria, F., Melo, R. M. C., Bazzoli, N., Rizzo, E. (2020). 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*, *190*, 110165.

Zhang, C., Shan, B., Tang, W., Wang, C., Zhang, L. (2019). Identifying sediment-associated toxicity in rivers affected by multiple pollutants from the contaminant bioavailability. *Ecotoxicology and environmental safety*, *171*, 84-91.

Zhou, Q., Yang, N., Li, Y., Ren, B., Ding, X., Bian, H., Yao, X., (2020). Total concentrations and sources of heavy metal pollution in global river and lake water bodies from 1972 to 2017. *Global Ecology and Conservation*, *22*, e00925.

2. Objetivos

Os principais objetivos deste estudo são: I - propor uma avaliação de risco ecológico (ARE) baseada nas concentrações de metais presentes nos sedimentos e na água, com base em dados disponíveis na literatura; II - descrever uma nova espécie de ostracode nativa da Amazônia, bem como seu ciclo de vida; III - realizar experimentos ecotoxicológicos agudos com as espécies aqui descritas, a fim de compreender sua sensibilidade a diferentes metais, tanto isoladamente quanto em misturas; IV - verificar, por meio de diferentes biomarcadores, se concentrações ambientalmente relevantes são capazes de induzir estresse e dano oxidativo.

2. Objectives

This study's main objectives are to: I - propose an ecological risk assessment (ERA) based on the concentrations of metals present in sediments and water, based on data available in the literature; II – describe a new species of ostracod native to the Amazon, as well as its life cycle; III - carry out acute ecotoxicological experiments with the species described here, in order to understand its sensitivity to different metals, both in isolation and in mixtures; IV - verify, through different biomarkers, whether environmentally relevant concentrations are capable of inducing stress and oxidative damage.

3. Hipóteses

I – As concentrações de metais levantadas em estudos disponíveis na literatura resultarão em elevado risco ecológico para os ecossistemas aquáticos da Amazônia;

II – A espécie descrita neste estudo pertencerá ao gênero *Strandesia*, resultado de uma convergência evolutiva entre outras duas espécies. Suas características ecológicas a favorecem para ser utilizada em estudos ecotoxicológicos;

III – O ostrácode *Strandesia rondoniensis* possui sensibilidade similar a outras espécies de ostrácodes testadas aos mesmos metais, e a combinação entre os metais resultará em efeitos sinérgicos;

IV – Concentrações Ambientalmente relevantes dos metais Cu, Cd, Hg e Zn resultarão em alterações bioquímicas levando a Stresse e dano oxidativo;

3. Hypotheses

I – The concentrations of metals raised in studies available in the literature will result in a high ecological risk for the aquatic ecosystems of the Amazon;

II – The species described in this study belongs to the genus Strandesia, the result of an evolutionary convergence between two other species. Its ecological characteristics favor it for use in ecotoxicological studies;

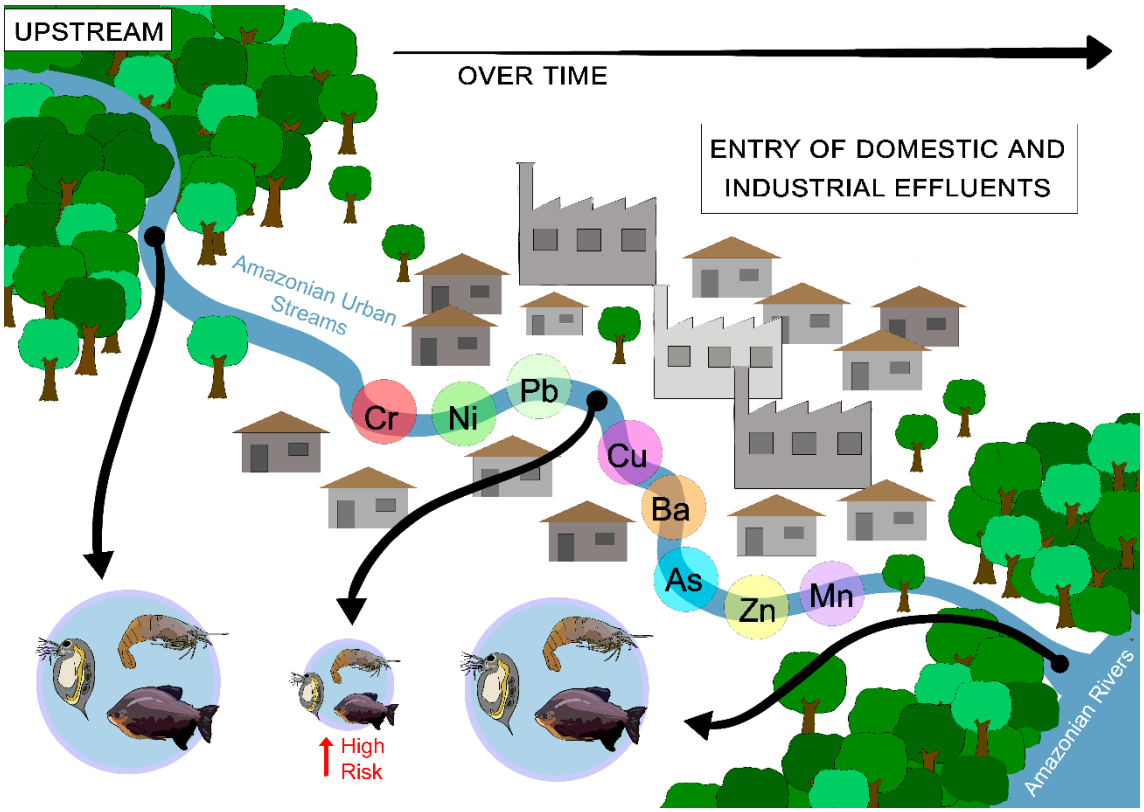
III – The ostracod *Strandesia rondoniensis* has similar sensitivity to other ostracod species tested to the same metals, and the combination between the metals will result in synergistic effects;

IV – Environmentally relevant concentrations of the metals Cu, Cd, Hg and Zn will result in biochemical changes leading to stress and oxidative damage;

Chapter 1 - Ecological risk assessment for metals in sediment and waters from the Brazilian Amazon region

Published in Chemosphere DOI: https://doi.org/10.1016/j.chemosphere.2023.140413

Graphic Abstract



Ecological risk assessment for metals in sediment and waters from the Brazilian Amazon region

Abstract

Pollution by metals is a matter of concern around the world. In recent decades, the high population growth in urban centers has significantly magnified the entry of these pollutants into aquatic ecosystems. The Amazon region, intense migratory flow, gold mining, and industrialization have been considered the main driving forces for increasing metal pollution. Thus, the main aim of this study is to conduct, for the first time, an Ecological Risk Assessment (ERA) based on metal concentrations measured in the sediment and water of several aquatic environments from the Amazon basin, based on the risk quotient values (RQ = measured environmental concentration - MEC/predicted no effect concentration - PNEC). In addition, the metal contamination factor (CF) was estimated. Although metal concentrations in water were generally low, these values were far above the limits established by current national legislation in many areas, showing higher concentrations for the metals Co, Pb, Cr, Cu, and Ni. Concentrations of Mn, Cu, Ba, Pb, Co, Ni, Cr, Zn, Cd, and As were especially high in the sediment for several evaluated environments. The ERA for the water compartment revealed that 56% of the studied areas presented high risk (RQ>1) for aquatic biota. In the sediment, 66% of the sites presented a high risk and 40% medium risk (RQ = 0.1-1). The CF indicated that 49% of the sampling points had high contamination and only 24%, had low contamination. These results reveal that monitoring studies in the Amazon region, provides important information so that public policies for the preservation of water resources can be strengthened in the Amazon.

Keywords: Contamination Factor; Freshwater Pollution; Legal Amazon; Metal Ecotoxicology; Risk Quotient.

1. Introduction

Pollution of water resources by potentially toxic metals is a reality worldwide (Viana et al., 2021). The natural entry of metals into aquatic ecosystems is usually associated with weathering of rocks, volcanism, rock formation, and erosion (Tchounwou et al., 2012; Mishra et al., 2019). Anthropogenic sources include mining, manufacturing, fertilizers, pesticides in agricultural areas, and waste disposal (Zhou et al., 2020). A very recent and comprehensive review study on the impact of mining activities on metal contamination showed that this is one of the main pollution problems of sediments and freshwater in the Amazon nowadays (Moulatlet et al., 2023). In this context, the Brazilian Amazon, which is home to the greatest biodiversity on the planet, with many species restricted to the biome (Rico et al., 2022), is not free from hazardous metal effects.

The Legal Amazon area represents approximately 60% of the Brazilian territory (Ribeiro et al., 2017). Territorial development in the States that contemplate the Amazon occurred quickly and in a disorderly manner, mainly between the 1960s and 1970s, when the Brazilian military government encouraged mineral exploration, agribusiness, highways, hydroelectric plants, and other large undertakings in the Amazon (Rico et al., 2022). The result of these public policies led to an intense migratory flow to the region (Matos, 2005; Barbieri, 2007). The consequent increasing population, agricultural and industrial growth throughout the Amazon has caused changes in land occupation patterns in the region (Souza, 2000; Rico et al., 2022). In general, about 18.6 million people inhabit the Amazon region (IBGE, 2020), and 80% of these inhabitants are concentrated in large urban areas (Castro et al., 2019; Albert et al., 2023). This has resulted in high deforestation rates (Cruz et al., 2021; Lapola et al., 2023) and the multiplication of pollution sources throughout the biome (Capparelli et al., 2020; Dos Santos et al., 2023).

One of the major pollution sources is untreated sewage discarded directly into the environment. According to the survey carried out by Instituto Trata Brasil (2022), the northern region of Brazil, which includes most of the legal Amazon, has a lack of basic sanitation, with approximately 88% of the population does not have access to sewer service. The same study also indicated that four capitals in the region were among the ten worst in the ranking, i.e., Macapá (AP), Porto Velho (RO), Belém (PA), and Rio Branco (AC). Untreated sewage may have several chemicals in its composition, including heavy metals, which could be partially removed with proper treatment (Tonani, 2008).

The sum of these pollution factors usually causes considerable concern among the scientific community, as urbanization and industrialization in large urban centers have brought high concentrations of metals into aquatic ecosystems worldwide (Khan et al., 2011; Ali et al., 2019). The metals are usually classified into two groups, essential: e.g., Fe, Zn, and Cu (Gupta, 2018) and non-essential: e.g., Cd, Pb, and Hg (Koller and Saleh, 2018). In low concentrations, essential metals are important for metabolic processes in different organisms. However, at high concentrations they become toxic (Rosabal et al., 2015). Therefore, the entry of high concentrations of metals represents a loss of integrity of any aquatic ecosystem, compromising the quality of water resources (Bernalte et al., 2020). The toxicity of metals varies according to the chemical speciation and availability of the metal, and by environmental conditions such as electrical conductivity, pH, and water hardness (Expósito et al., 2017).

Many studies conducted in the Amazon region have shown that contamination of water resources by effluents with high metal concentrations has evidenced the bioaccumulation in muscle tissues of fish and alligators, as well as in aquatic invertebrates (de Souza, 2016; Silva et al., 2022; Gomes et al., 2020; Silva et al., 2023) with consequent biomagnification for the entire food chain (Zafarzadeh et al., 2018; Lima et al., 2023). Thus, there is currently concrete evidence that the entry of these metals into aquatic ecosystems represents a potential threat to aquatic biota (Goix et al., 2014; Leveque et al., 2014; Rocha et al., 2018; Vatandoost et al., 2018; Ali et al., 2019; Dos Santos et al., 2023) and human health (Lino et al., 2019; Gomes et al., 2020). However, there are still few studies that assess the environmental risks associated with the pollution of Amazonian aquatic ecosystems by metals.

Based on the above, the present study aims to (i) carry out, for the first time, an Ecological Risk Assessment (ERA) for metals in water and sediment from different regions of the Amazon, allowing reflections on the ecological consequences resulting from the disorderly growth of Amazonian cities and (ii) calculate contamination factor (CF) based on the metal concentrations found in the sediment.

2. Methods

2.1 Study focus

Metal concentrations for sediment and water were collected through a literature search of studies conducted between 1990 and 2023. When there was more than one value for the same metal in the same study or different seasons, the arithmetic mean was calculated. The search was carried out using the databases Google Scholar, Scientific Electronic Library Online (SciELO), Periodicals of Capes, and Web of Science. The following keywords were used: “metals”, “heavy metals”, “sediment”, “water”, “Amazon”, and “aquatic ecosystems”. The inclusion criteria were: i) Full Articles, Short Communications, Theses, or dissertations, which objective was to quantify metals for both sediment and water exclusively in the Brazilian Amazonian region; ii) full text available; iii) language in Portuguese or English and iv) use and description of a standardized quantification technique. Both articles published in indexed journals and studies published in the “grey literature” (Thesis and Dissertation) were included since many data have not yet been published or are still in this process (Brophy and Bawden, 2005; Falagas et al., 2008; Delgado and Repiso, 2013). Since each database takes into account an index for classification, the combination of them forms a more robust methodological framework with less bias (Mongeon and Paul-Hus, 2016). Incomplete articles and studies that did not adhere to the selection criteria listed above were not included. Figure 1 highlights the locations selected for the study. The complete dataset obtained is presented as supplementary material (Tables S1, S2, and S9).

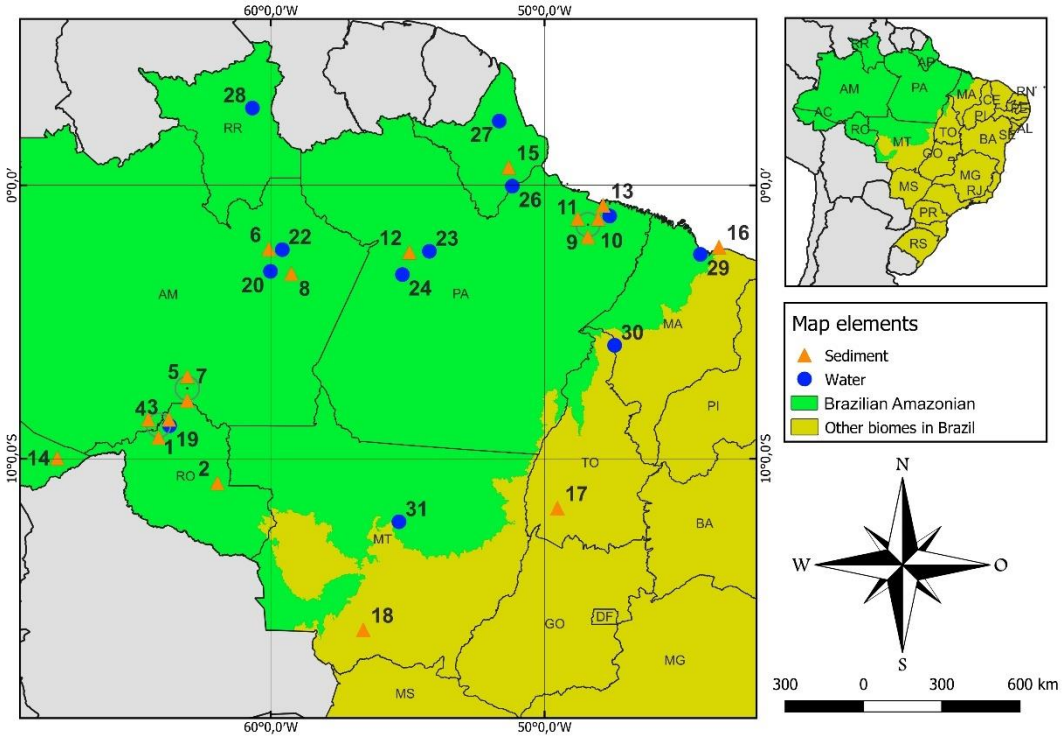


Figure 1 - Map of studies that quantified metals in sediment and water for different regions of the Amazon. For sediment we used the following studies: 1 – Streams Tanques (Igarapé); 2 - Jaru Biological Reserve; 3 – Madeira River; 4 - Tributaries of the Madeira River; 5 - Lake Puruzinho; 6 - Taruma-Açu basin; 7 - Urban Streams of Humaita; 8 - Amazon River; 9 - Guajara Bay; 10 - Maguari River; 11 - Lake black water and Bologna; 12 - Metropolitan Region of Santarém; 13 - Amazon Estuary; 14 - Acre River; 15 - Amapari River; 16 - São Marcos Bay; 17 – Formoso River; 18 - Bento Gomes River. The locations for water samples were: 19 – Porto Velho - urban streams; 20 - Negro River; 21 - Students' Basin; 22 - Taruma-Açu Basin; 23 - Metropolitan Region of Santarem; 24 - Tapajos River; 25 - Nascent Cumaru Basin; 26 - Amazon River; 27 - Cassipore River; 28 - Branco Basin River; 29 - São Marcos Bay; 30 - River do Sono and Araguaia; 31 - Caiabi River.

2.2 Ecological Risk Assessment

2.2.1 Species sensitivity distributions

Ecotoxicological information was collected to allow the calculation of the environmental risks associated with the metal concentrations obtained for the water and sediment compartments. To this end, acute toxicity data (LC_{50} and EC_{50}) for aquatic species of different trophic levels, except primary producers, were collected from the US-EPA ECOTOX database (https://cfpub.epa.gov/ecotox/), using the filter for “Cadmium”, “Copper”, “Zinc”, “Manganese”, “Arsenic”, “Cobalt”, “Nickel”, “Chromium”, “Barium”, “LC_{50}”, and “EC_{50}”. This was complemented through a literature search using Web of Science, Scopus, and Science Direct databases, using as a filter the keywords “Ecotoxicological Assessment”, “LC_{50}”, “metals”, and the scientific name of selected aquatic vertebrates and invertebrates. When different values were found for the same species concerning the same tested metal and experimental condition, the geometric mean of toxicity data was used. All references used for constructing species sensitivity distributions (SSDs) curves are presented as Supplementary Material (Table S3). The SSDs curves (Figure S3), as well as the derivation of the hazard concentration for 5% of the species (HC_5) and their 95% confidence limits, were obtained using the ETX software, version 2.3 (Van Vlaardingen et al., 2004) and the graphs were generated through the method outlined in Thorley and Schwarz (2018).

2.2.2 Water

The ERA for the water compartment was performed by deriving risk quotients (RQ), i.e. the ratio between the environmental concentrations measured in water

(MEC_{water}), obtained as described above, and the predicted no-effect concentration for water ($PNEC_{water}$). PNEC values were calculated by dividing the HC_5 value, obtained from the SSDs curves, with an assessment factor (AF = 5). This AF value was selected considering the degree of uncertainties associated with the number of ecotoxicological information on different taxonomic groups available for the construction of SSD curves (EFSA, 2002). The RQ values were interpreted according to the scale: Low risk (RQ <0.1), Medium risk (RQ ranging 0.1–1), and high risk (RQ>1) (Lu et al., 2022; Yan et al., 2022).

2.2.3 Sediment

The RQ values for the sediment compartment were also calculated as the ratio between MEC and PNEC. The PNEC was derived from the extrapolation of $PNEC_{water}$ to each metal using the equilibrium partitioning method (Equation 1) described in EFSA (2002).

$$PNEC_{sed} = \frac{k_{susp-water}}{RHO_{susp}} PNEC_{water}.1000 \qquad \text{Eq. 1}$$

The partition coefficient of suspended matter-water ($k_{susp\text{-}water}$) for each studied metal was obtained from USEPA (2005), except for manganese which value was obtained from Sheppard et al. (2009). The bulk density of wet suspended matter (RHOsusp) was calculated according to Equation 2 (EFSA, 2002):

$$RHO_{susp} = Fsolid_{sed}.RHOsolid + Fwater_{sed}.RHOwater \qquad \text{Eq. 2}$$

The fraction of solids ($Fsolid_{sed}$) and water ($Fwater_{sed}$) in sediment and the density of the water phase (RHO_{water}) were obtained from EFSA (2002). The solid phase density (RHO_{solid}) for the soil in the study area was derived from Ajayi et al. (2009).

2.3 Metal Contamination Factor in the Sediment

The contamination factor CF is used to evaluate sediment contamination by heavy metals and is calculated according to equation 3 (after Hakanson, 1980).

$$CF = \frac{C_n}{C_b} \qquad \text{Eq. 3}$$

Where C_n = levels of metals used in the study, C_b = average of metal reference values (In mg/kg: Co = 2.1; Mn = 29.1; Cr = 192.6; Cu = 2.7; Ni = 7.2; Ba = 2.1; Zn = 2.7; (Rebêlo et al., 2020) and Cd = 0.1; Pb = 4.2 (Do Nascimento et al., 2018) and As = (15.9) (Silva-Junior et al., 2019). The scales used to categorize the calculated contamination factors are presented in Table S4 (after Hakanson, 1980).

2.4 Data Analysis

A PCA analysis was applied to verify the spatial distribution of metals in water and sediment. The analyses were carried out in Software R (Version 4.1.3, 2022) with the application of R studio (2023.03.1).

3. Results and Discussion

3.1 Metal concentrations in water

The survey of metal concentrations in water reveals that the metals Co (9.53 mg L^{-1}), Cd (7.66 mg L^{-1}), Pb (3.81 mg L^{-1}), Cr (2.69 mg L^{-1}), Ni (2.55 mg L^{-1}), and Cu (0.62 mg L^{-1}) were detected in the highest concentrations in the studies evaluated. Considering all studied metals, about 44% of the evaluated areas had concentrations above the limits established by the National Environment Council - CONAMA (resolution 357) (Brasil, 2015) for the most restrictive classification regarding the preponderant use of water that presents quality standards (Class I Waters). Only Ba and Mn did not exceed the limits established by law. Detailed data can be viewed in supplementary Table S1. In the PCA analysis, PC1 accounted for 42% of data variability and PC2 21% (Figure S1). Most sampling sites were grouped in PCA, thus indicating a spatial homogeneity, except for the Manaus (Amazonas State) and Lourenço (Amapá State) sites that were separated and directly influenced by concentrations of Cu, Pb, Cd, Ni, and Co to the Manaus area and Zn and Cr to the Lourenço area.

Although the metal concentrations listed in this study are at some points below the limits established by legislation (CONAMA 357/05), the metals found in water are normally free metallic materials, making them bioavailable to be incorporated by aquatic organisms (Balistrieri et al., 2020; Mebane et al., 2020). The continuous entry and excess of metals in water can increase the risk to organisms, as potentially toxic metals can cause

hazardous effects such as carcinogenicity, genotoxicity, and changes in the endocrine system (Hussain et al., 2019; Maurya et al., 2019), generating risk to entire aquatic ecosystems.

3.2 Metal concentration in the sediment

The metal concentrations obtained for the sediment compartment revealed that all metals reached values above the limits established by CONAMA Resolution 454 (BRASIL, 2012) in some sampling points, with higher concentrations for Mn (1856.8 mg kg^{-1}), Cu (537.06 mg kg^{-1}), Ba (309 mg kg^{-1}), Pb (221.6 mg kg^{-1}), Co (160.3 mg kg^{-1}), Ni (152.25 mg kg^{-1}), Cr (143.3 mg kg^{-1}), Zn (129.1 mg kg^{-1}), Cd (5.4 mg kg^{-1}) and As (2.8 mg kg^{-1}). The areas with the highest concentrations were the Tarumã-Açu basin - AM (Santana and Barroncas, 2007), Água Preta Lake, and Bolonha - PA (Oliveira et al., 2018), Igarapé dos Tanques - RO (Dos Santos et al., 2012) and Amapari River - AP (da Silva et al., 2013) (Table S2). Despite that, only the Tarumã-Açu data (Manaus) were separated by the other sampling stations by the PCA analysis (PC1 42% and PC2 21% of data variability) by the PC1, mainly due to the higher concentrations of As, Ba, Pb, Co, Ni, Cu, and Zn. The Amazon river (Manaus) was also separated by the PC2 due to the high concentrations of As and Ba. The other stations were most homogeneously grouped (Figure S2).

The high concentrations in the sediment suggest that using soil and the expansion of large urban centers are responsible for the degradation of aquatic ecosystems, mainly after the arrival of several waves of migrants in the region, which resulted in a population increase of 15% over a period of 20 years for the entire Amazon region (IBGE, 2010, 2020). Albert et al. (2023) found that the rate of anthropic interference is affecting Amazonian ecosystems on scales never seen before, causing fast degradation of terrestrial and aquatic ecosystems. This scenario is occurring in other small, medium, and large cities located in the Amazon region. It is therefore crucial to understand the environmental risks associated with the lack of urban planning in the cities and extend discussions about the consequences of the expansion of Amazonian urban centers to biodiversity conservation, in general. Thus, the anthropic pressure increased on urban aquatic ecosystems, mainly due to the discharge of high volumes of untreated domestic and industrial effluents, which directly flow into the aquatic environments. Thus, sediments are one of the main sinks and/or sources of metals in the water column (Singh et al., 2005;

Kumar et al., 2019). High metal concentrations in the sediment occur mainly because in aquatic environments, metals, metal ions or metalloids are available in different forms, allowing them to be adsorbed to particulate matter in suspension quickly and subsequently incorporate into bottom sediments (Filgueiras et al., 2004). Furthermore, sediments have a high capacity to incorporate various toxic substances, with more than 99% of pollutants consumed ending up in the sedimentary compartment (Förstner, 1995).

3.3 Contamination Factor

The CF results (Figure 2 and Table S5) show that about 49% of the analyzed points present high contamination (CF≥6), 12.72% moderate contamination (3≤CF<6), 13.63% considered contaminated (1≤CF<3) and 24.54% low contamination (CF<1). The average of CF values for each metal shows that the highest values were observed for Co (2157), Cu (998), Pb (564.4), Ba (58.1), Zn (25.6), Cd (22.9), Mn (14.2), Cr (13.3), Ni (4.2) and As (1.2) (Figure 2). Arisekar et al. (2022) reported lower CF values in a sediment sample from the Thamirabarani River: Cd (0.32 - 7.56), As (0.17 - 0.48), Pb (0.04 - 0.12), Co (0.12 - 2.38), Zn (0.14 - 0.87), Ni (0.23 - 0.83), Cr (0.01 - 0.21), Cu (0.01 - 1.08) and Mn (0.01 - 0.06). Kusin et al. (2018) did not observe contamination in sediment samples collected in the Pengerang area, Malaysia, for the metals Mn, Fe, Al, Zn, Cr, Co, Cd, and Sr, as the CF values were <1 for most of the studied sites. Maanan et al. (2015) reported CF values for Cd (7.9), Cr (1.2), Cu (3.3), Ni (2.4), Pb (6.3), and Zn (5.3) for Nador lagoon – Morocco. The comparisons made here reinforce the critical situation verified in this study for the Amazon biome, with high levels of contamination for several regions of Amazonia. Thus, public policies for monitoring and preserving water resources need to be urgently improved. The most contaminated areas (Acre, Rondônia, Amazonas, Pará, and Roraima States) were in the Northern region of Brazil (Figure 1), which has a low sewage system coverage, as previously described, reinforcing that the sanitation precarity has been contributing to the freshwater degradation.

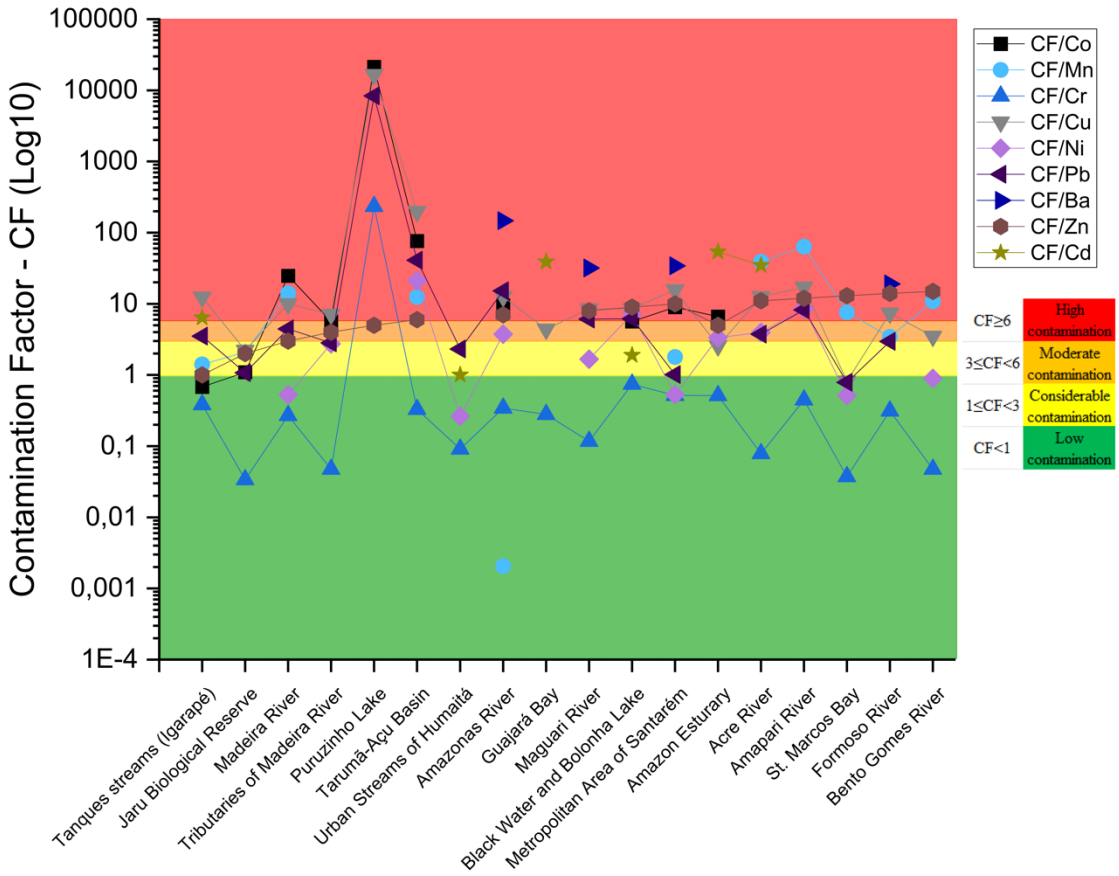


Figure 2 - Values of contamination factor obtained for sediments from waterbodies of different regions in the Amazon. The risk scale can be seen in table S4.

Thus, the results presented allow us to discuss the risks associated with sedimentary contamination in this biome and the factors associated with this process. Nascimento et al. (2021) verified that the unplanned urban expansion that occurred in recent decades is one of the main factors for the entry of untreated effluents into the Tapajós River. According to these authors, the continuous entry of effluents can cause a serious reduction in the water quality of the Tapajós River and streams in urban areas of the Amazonian biome. Ferreira et al. (2021) found that the water quality parameters were completely changed, mainly in the urban areas of Manaus-AM, and attributed these changes to untreated sewage released directly into urban streams with the presence of dumps and household waste. Rico et al. (2021) found that urban areas are primarily responsible for chemical contamination. According to the authors, some mixtures had up to 40 different compounds and many of them were above the values established by law. Cabrera et al. (2023) concluded that urban areas in the Ecuadorian Amazon significantly alter water quality parameters affecting macroinvertebrate communities with 20% and

68% of points with acute and chronic ecological risk. Galarza et al. (2022) point out that the emission and mobilization of metals and metalloids in different regions of the Amazon come from mining activities, representing a high risk to human health. In turn, Moulatlet et al. (2023) recently presented a review of metals in the Amazon and found that mining activities directly contribute to the entry of metals into water bodies in the Amazon.These data reinforce the precariousness of basic sanitation, added to different anthropic activities in this region, contributing to the pollution of freshwaters. In fact, in the North region of Brazil, where most of the Brazilian legal Amazon is located, only 14% of the population had access to the sewage network in 2021 (Brasil, 2023).

3.5 Ecological Risk Assessment

3.5.1 Water

The HC_5 and HC_{50} values, as well as the SSDs curves, are presented as Supplementary Material (Table S6 and Figure S3). The ecological risk assessment was performed based on the hazardous concentrations affecting 5% (HC_5) and the MEC values presented in this study. The analysis showed that 56% of the sites had RQ values higher than one. Although the metal concentrations in the water samples for some sites were below the limits established by law, the risk assessment indicated a high ecotoxicological risk for aquatic vertebrates and invertebrates, as the RQ values for all metals, except Ba, were greater than one for some locations, representing a high risk for aquatic biota. The highest RQ values were estimated for Cd - 216.7; Cu - 170.2; Cr - 55.73; Co - 30.2; Ni - 17.02; As - 1.62; Mn - 1.37; Zn - 0.92, and Ba - 0.006. Considering the data obtained, the presence of these metals poses a high risk to the biota of all sampled sites (Figures 3, 4, and Table S7).

Sensitivity data available in the literature show that the acute sensitivity ($LC_{50,48h}$) of the species *Daphnia magna, D. pulex,* and *Ceriodaphnia reticulata* for Cr are 0.118, 0.068 and 0.066 mg L^{-1}, respectively, whereas the $LC_{50,48h}$ for Cu are 0.054, 0.053 and 0.017 mg L^{-1}, respectively (Mount and Norberg,1984). The $LC_{50,48h}$ of *D. similis* for Cr is 0.021 mg L^{-1} (Sotero-Santos et al., 2007). Freitas and Rocha (2014) reported a significant reduction in the fecundity of the cladoceran *Pseudosida ramosa* when exposed to concentrations of 0.01 mg Cr L^{-1}. Belanger and Cherry (1990) found that Zn concentrations of 0.05 mg L^{-1} significantly reduced the reproduction of the cladoceran *C.*

dubia. Sadeq and Beckerman (2019) pointed out that 0.025 mg Cu L^{-1} led to drastic changes in the reproduction of *D. longispina* and *Moina mongolica*. Real et al. (2003) show that Cu in low concentrations affected the biomass and reproduction of the snail *Stagnicola vulnerata*. Arthur and Leonard (1970) reported that 0.028 mg L^{-1} of Cu was sufficient to halter the growth of the mollusk *Physa integra*. Dorgelo et al. (1995) verified that there was inhibition of the growth rate of the snail *Potamopyrgus jenkinsi* when exposed to Cu concentrations lower than 0.01 mg L^{-1}. For fish, metals can cause neurotoxic effects by causing malformation in different organs, affecting their survival and growth (Ali et al., 2019). In addition, studies by Chen et al. (2022) indicated an increase in the toxicity of Cd and Cu metals when exposed in combination with the ostracod *Cypridopsis vidua*. More recent studies presented by Lima et al. (2023) verified synergism in binary exposures of mixtures for the metals Cu, Hg and Mn, on the ostracod *Strandesia trispinosa*, according to the authors, the effects were aggravated mainly when Cu predominated in the mixture. Moreover, Gu et al. (2022) found an average probability of 54.6% of toxic effect to the aquatic biota for sediment samples from the surface of Zhelin Bay contaminated with different metals and pesticides. Therefore, the entry of multiple pollutants into aquatic ecosystems represents an even greater risk, since the interaction of pollutants in aquatic ecosystems, in many cases, increases the toxicity of metals.

3.5.2 Sediment

Based on the risk assessment of the sediment compartment, only the metals As, Pb, Cu, and Mn presented averages of RQ lower than one, although for some specific sites values greater than one were denoted. All values for the remaining metals analyzed were in the high risk scale to the biota, with RQ values of 10 to 100 in several cases for most sites analyzed. The highest RQ values obtained for the different metals were: Ni (59.4), Zn (33.5), Co (12.2), Cr (5.9), Ba (5.7), As (1.5), Mn (0.9), Cu (0.6), Cd and Pb (0.4) (Figure 3B and table S8). The analysis also reveals that a total of 66.32% of the sampled locations presented high risk (RQ>1), 39.79% Medium Risk (RQ ranging 0.1 – 1), and only 12.2% of the points had low risk (RQ<0.1).

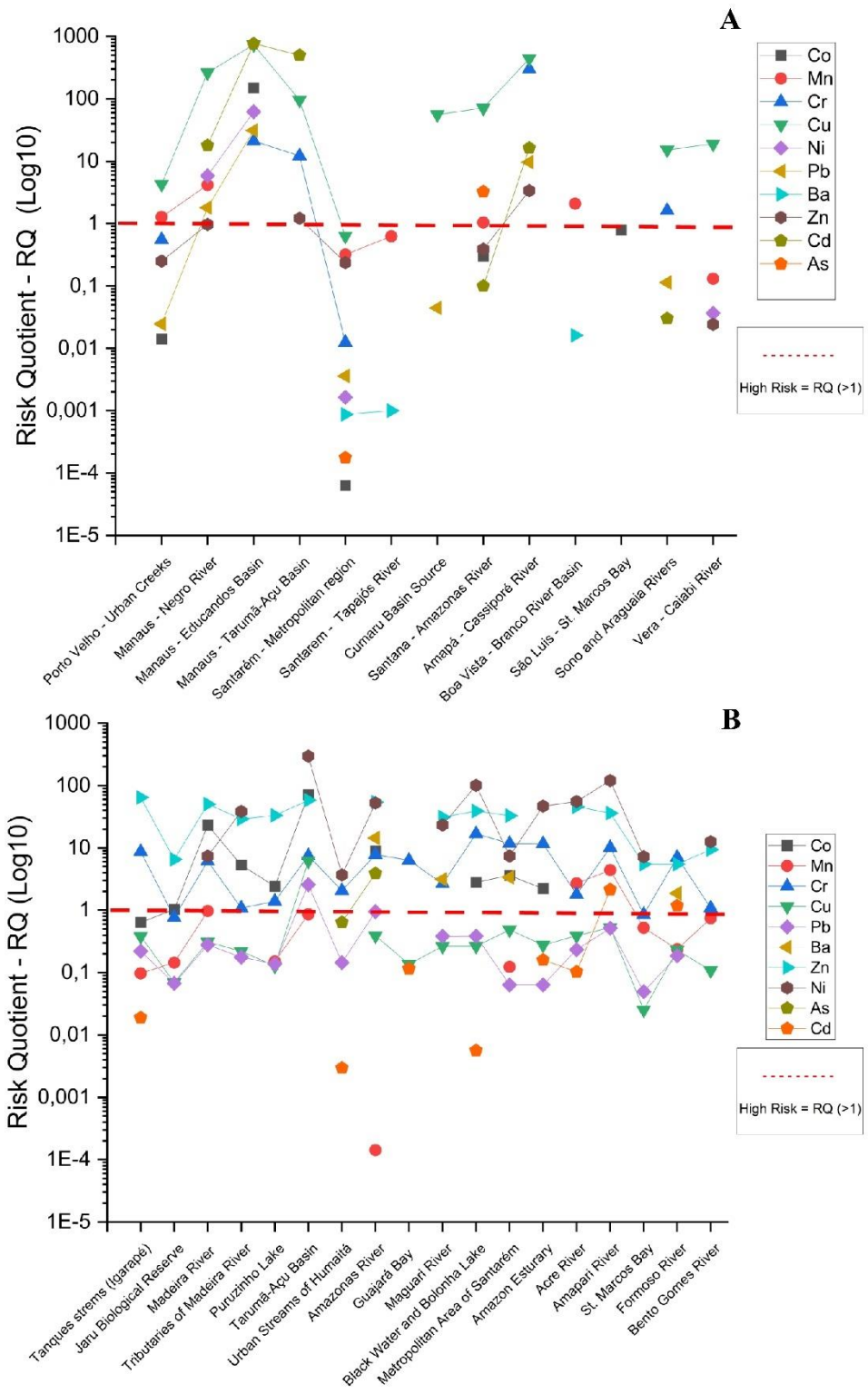


Figure 3 - Ecological risk analysis for metals analyzed in water and sediment, expressed as ecological risk quotient (RQ), based on the hazard concentration for 5% (HC5) (PNEC in mg L^{-1}) for all sites sampled in the water (A) and sediment (B). The QR values were interpreted according to the scale: Low risk = QR (<0.1), Medium risk = QR (0.1–1) High risk = QR (>1).

The majority of metal concentrations in the Amazonian river basin sediments evaluated for contamination and risk for aquatic biota as a whole showed evidence of high risk for the aquatic biota, which is a matter of concern for the Amazonian aquatic ecosystems. Sediment concentrations for almost all metals (MEC_{sed}) were above the estimated PNEC values for this compartment. Lima et al. (2019) found that different species of ostracods showed high sensitivity to different metals. Suedel et al. (1997) reported that low concentrations of different metals caused serious negative effects on different aquatic invertebrates. Gazonato Neto et al. (2019) verified the high sensitivity of benthic oligochaetes *Allonais inaequalis* and *Dero furcatus* to Cu and Cd metals. This high metal load in the sediment represents a potential risk for aquatic ecosystems the more since metals have high toxicity even at low concentrations (Vatandoost et al., 2018; Ferrey et al., 2018), have high persistence in the environment (Mishra et al., 2019), as well as easy bioaccumulation and biomagnification through the food chains (Valdés et al., 2014; Liu et al., 2018; Vatandoost et al., 2018). Thus, the sum of these factors can cause serious damage to aquatic organisms that live both in the water column and the sediment (Barałkiewicz et al., 2014).

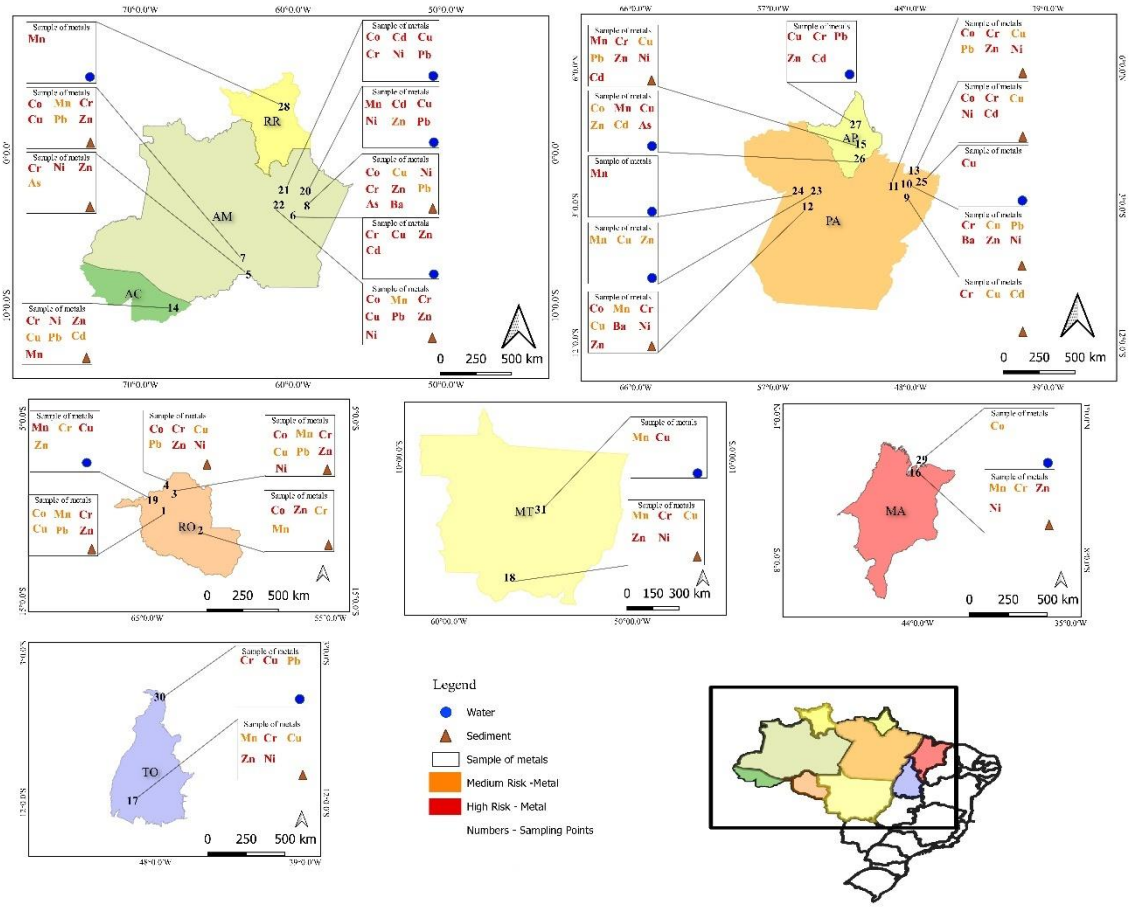


Figure 4 - Map of medium and high risks for all samples of metals in sediment and water at all studied locations. For sediment we used studies in the following water resources: 1 – Streams Tanques (Igarapé); 2 - Jaru Biological Reserve; 3 – Madeira River; 4 - Tributaries of the Madeira River; 5 - Lake Puruzinho; 6 - Taruma-Açu basin; 7 - Urban Streams of Humaita; 8 - Amazon River; 9 - Guajara Bay; 10 - Maguari River; 11 - Lake Black water and Bologna; 12 - Metropolitan Region of Santarém; 13 - Amazon Estuary; 14 - Acre River; 15 - Amapari River; 16 - São Marcos Bay; 17 – Formoso River; 18 - Bento Gomes River. The locations for water samples were: 19 – Porto Velho - urban streams; 20 - Negro River; 21 - Students' Basin; 22 - Tarumã-Açu Basin; 23 - Metropolitan Region of Santarem; 24 - Tapajos River; 25 - Nascent Cumaru Basin; 26 - Amazon River; 27 - Cassipore River; 28 - Branco Basin River; 29 - São Marcos Bay; 30 - Sono River and Araguaia; 31 - Caiabi River.

4. Conclusions

Based on the results of this study, it can be concluded that the continuous entry of domestic and industrial effluents into the Amazonian aquatic ecosystems causes high concentrations of metals in the sediment. This represents a warning sign about the temporal accumulation of these compounds. As for the ERA of the water compartment, our data showed that Co, Pb, Cr, Cu, and Ni posed the highest risks while in the sediment Mn, Cu, Ba, Pb, Co, Ni, Cr, Zn, Cd, and As were the most dangerous. Besides, the contamination factor indicated that most of the studied sites presented high contamination. Thus, public policies must be designed for the ordering and growth of urban centers in the region as high risks were reported. Finally, metal concentrations in water and sediments pose a potential threat, as most metallic pollutants have high persistence, bioaccumulate and biomagnify across the food web. Thus, this study may contribute to increasing our knowledge on the risks of potentially toxic metals present in domestic and industrial urban efluents which are nowadays a threat to the aquatic biota in both sediment and water of the intrincated aquatic network at human occupied regions of the Amazon biome.

References

Albert, J. S., Carnaval, A. C., Flantua, S. G., Lohmann, L. G., Ribas, C. C., Riff, D., Nobre, C. A., 2023. Human impacts outpace natural processes in the Amazon. *Science*, *379* (6630).

Ali, H., Khan, E., Ilahi, I., 2019. Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry*, 1–14.

Ajayi, A. E., Junior, M. D. S. D., Curi, N., Junior, C. F. A., Souza, T. T. T., Junior, A. V. I., 2009. Strength attributes and compaction susceptibility of Brazilian Latosols. *Soil and Tillage Research*, *105* (1), 122-127.

Arthur, J. W., Leonard, E. N., 1970. Effects of copper on *Gammarus pseudolimnaeus*, *Physa integra*, and *Campeloma decisum* in soft water. *Journal of the Fisheries Board of Canada*, *27* (7), 1277-1283.

Arisekar, U., Shakila, R. J., Shalini, R., Jeyasekaran, G., Keerthana, M., Arumugam, N., Perumal, K., 2022. Distribution and ecological risk assessment of heavy metals using geochemical normalization factors in the aquatic sediments. *Chemosphere*, *294*, 133708.

Barbieri, A. F., 2007. Towns in the Jungle: Exploring Linkages between Rural-Urban Mobility, Urbanization, and Development in the Amazon Center for Regional Development and Planning (CEDEPLAR), Federal University of Minas Gerais (UFMG), Brazil, and Richard E . Bilsborrow (North).

Belanger, S. E., Cherry, D. S., 1990. Interacting effects of pH acclimation, pH, and heavy metals on acute and chronic toxicity to *Ceriodaphnia dubia* (Cladocera). *Journal of Crustacean Biology*, *10* (2), 225-235.

Bernalte, E., Arévalo, S., Pérez-Taborda, J., Wenk, J., Estrela, P., Avila, A., Di Lorenzo, M., 2020. Rapid and on-site simultaneous electrochemical detection of copper, lead and mercury in the Amazon river. *Sensors and Actuators B: Chemical*, *307*, 127620.

BRASIL - CONSELHO NACIONAL DO MEIO AMBIENTE (CONAMA). Resolução nº 357, de 17 de março de 2005. Ministério do Meio Ambiente.

BRASIL - CONSELHO NACIONAL DO MEIO AMBIENTE (CONAMA). Resolução n. 457, de 13 de maio de 2012. Ministério do Meio Ambiente.

BRASIL - Lei nº 12.651, de 25 de maio de 2012. Dispõe sobre a proteção da vegetação nativa; altera as Leis nos 6.938, de 31 de agosto de 1981, 9.393, de 19 de dezembro de

1996, e 11.428, de 22 de dezembro de 2006; revoga as Leis nos 4.771, de 15 de setembro de 1965, e 7.754, de 14 de abril de 1989, e a Medida Provisória no 2.166-67, de 24 de agosto de 2001; e dá outras providências.

BRASIL., 2020. Instituto Brasileiro de geografia e Estatística (IBGE). *Censo demográfico, 2020*, 11.

BRASIL., 2023. Ministério da Integração e do Desenvolvimento Regional. *Esgotamento Sanitário. Disponível em: https://www.gov.br/mdr/pt-br/assuntos/saneamento/snis/painel/es Acess in: 26/07/2023.*

Balistrieri, L. S., C. A., Mebane T. S. Schmidt., 2020. Timedependent accumulation of Cd Co, Cu, Ni, and Zn in natural communities of mayfly and caddisfly larvae: metal sensitivity, uptake pathways, and mixture toxicity. *Science of the Total Environment* 732, 139011.

Barałkiewicz, D., Chudzińska, M., Szpakowska, B., Świerk, D., Gołdyn, R., Dondajewska, R., 2014. Storm water contamination and its effect on the quality of urban surface waters. *Environmental monitoring and assessment*, 186, 6789-6803.

Brophy, J., Bawden, D., 2005. Is Google enough? Comparison of an internet search engine with academic library resources. In Aslib proceedings. *Emerald Group Publishing Limited*

Cabrera, M., Capparelli, M. V., Ñacato-Ch, C., Moulatlet, G. M., López-Heras, I., González, M. D., Rico, A. (2023). Effects of intensive agriculture and urbanization on water quality and pesticide risks in freshwater ecosystems of the Ecuadorian Amazon. *Chemosphere*, 139286.

Capparelli, M.V., Moulatlet, G.M., Abessa, D.M.S., Lucas-Solis, O., Rosero, B., Galarza, E., Tuba, D., Carpintero, N., Ochoa-Herrera, V., Cipriani-Avila, I., 2020. An integrative approach to identify the impacts of multiple metal contamination sources on the Eastern Andean foothills of the Ecuadorian Amazonia. *Sci. Total Environ*. 709, 136088.

Castro, M.C., Baeza, A., Codeço, C.T., Cucunub´a, Z.M., Dal'Asta, A.P., De Leo, G.A., Dobson, A.P., Carrasco-Escobar, G., Lana, R.M., Lowe, R., Monteiro, A.M.V., Pascual,

M., Santos-Vega, M., 2019. Development, environmental degradation, and disease spread in the Brazilian Amazon. *PLoS Biol*. 17, 4–11.

Chapman, P. M., 2008. Environmental risks of inorganic metals and metalloids: a continuing, evolving scientific odyssey. *Human and Ecological Risk Assessment*, *14* (1), 5-40.

Chen, L., Huo, Z., Su, C., Liu, Y., Huang, W., Liu, S., Sui, Q., 2022. Sensitivity of Ostracods to U, Cd and Cu: The Case of Cypridopsis vidua. *Toxics*, 10 (7), 349.

Cruz, D. C., Benayas, J. M. R., Ferreira, G. C., Santos, S. R., Schwartz, G. 2021. An overview of forest loss and restoration in the Brazilian Amazon. *New Forests*, *52*, 1-16.

da Silva. D. T., Lafon. J. M., Corrêa. J. A. M. 2013., Distribuição de metais pesados e isótopos de Pb em sedimentos do rio Amapari. setor de Pedra Branca do Amapari–Porto Grande. Amapá. Brasil. *Boletim do Museu Paraense Emílio Goeldi-Ciências Naturais*. *8* (2). 177-203

da Silva Júnior, E. C., Martins, G. C., de Oliveira Wadt, L. H., Da Silva, K. E., de Lima, R. M. B., Batista, K. D., Guilherme, L. R. G., 2019. Natural variation of arsenic fractions in soils of the Brazilian Amazon. *Science of the total environment*, 687, 1219-1231.

de Sousa, E. A., Luz, C. C., de Carvalho, D. P., Dorea, C. C., de Holanda, I. B. B., Manzatto, Â. G., Bastos, W. R., 2016. Chromium distribution in an Amazonian river exposed to tannery effluent. *Environmental Science and Pollution Research*, 23, 22019-22026.

Delgado, E., and Repiso, R., 2013. El impacto de las revistas de comunicación: comparando Google Scholar Metrics, *Web of Science y Scopus*. Comunicar, 21 (41), 45-52.

Dos Santos, C. C. M., Nauar, A. R., Ferreira, J. A., da Silva Montes, C., Adolfo, F. R., Leal, G., Amado, L. L., 2023. Multiple anthropogenic influences in the para river (amazonia, Brazil): A spatial-temporal ecotoxicological monitoring in abiotic and biotic compartments. *Chemosphere*, 138090.

Dos Santos, J. P., de Souza, D. D. O., Miranda, M. R., Bastos, W. R., 2012. Studies of trace elements in the city of Porto Velho/Rondônia, Western Amazon. *Ecotoxicology and Environmental Contamination, 7* (2).

Do Nascimento, C. W. A., Lima, L. H. V., da Silva, F. L., Biondi, C. M., Campos, M. C. C., 2018. Natural concentrations and reference values of heavy metals in sedimentary soils in the Brazilian Amazon. *Environmental monitoring and assessment*, *190*, 1-9.

Dorgelo, J., Meester, H., van Velzen, C., 1995. Effects of diet and heavy metals on growth rate and fertility in the deposit feeding snail Potamopyrgus jenkinsi (Smith) (Gastropoda: Hydrobiidae). *Hydrobiologia* 316, 199-210.

Expósito, N., Kumar, V., Sierra, J., Schuhmacher, M., Papiol, G. G., 2017. Performance of *Raphidocelis subcapitata* exposed to heavy metal mixtures. *Science of The Total Environment*, *601*, 865-873.

EFSA. European Food Safety Authority, 2002. Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances. EFSA supporting publication: EN-784. 39 p.

Falagas, M. E., Pitsouni, E. I., Malietzis, G. A., Pappas, G., 2008. Comparison of PubMed, Scopus, web of science, and Google scholar: strengths and weaknesses. *The FASEB journal*, 22(2), 338-342.

Ferreira, S. J. F., Pinel, S., Ríos-Villamizar, E. A., Miranda, S. Á. F., Pascoaloto, D., Vital, A. R. T., da Cunha, H. B. (2021). Impact of rapid urbanization on stream water quality in the Brazilian Amazon. *Environmental Earth Sciences*, *80*, 1-16.

Ferrey, M. L., Hamilton, M. C., Backe, W. J., Anderson, K. E., 2018. Pharmaceuticals and other anthropogenic chemicals in atmospheric particulates and precipitation. *Science of the Total Environment*, *612*, 1488-1497.

Filgueiras, A. V., Lavilla, I., Bendicho, C., 2004. Evaluation of distribution, mobility and binding behaviour of heavy metals in surficial sediments of Louro River (Galicia, Spain) using chemometric analysis: a case study. *Science of the Total Environment*, *330*(1-3), 115-129.

Förstner, U., Salomons, W., Stigliani, W., 1995. Biogedynamics of pollutants in soils and sediments. *ed. W. Salomons and WM Stigliani*.

Freitas, E. C., and Rocha, O., 2014. Acute and chronic toxicity of chromium and cadmium to the tropical cladoceran pseudosida ramosa and the implications for ecotoxicological studies. *Environmental Toxicology*, *29* (2), 176-186.

Galarza, E., Moulatlet, G. M., Rico, A., Cabrera, M., Pinos-Velez, V., Pérez-González, A., Capparelli, M. V. (2023). Human health risk assessment of metals and metalloids in mining areas of the Northeast Andean foothills of the Ecuadorian Amazon. *Integrated Environmental Assessment and Management*, *19*(3), 706-716.

Gazonato Neto, A. J., Moreira, R. A., Lima, J. C. D. S., Daam, M. A., Rocha, O., 2019. Freshwater neotropical oligochaetes as native test species for the toxicity evaluation of cadmium, mercury and their mixtures. *Ecotoxicology*, *28*, 133-142.

Goix, S., Lévêque, T., Xiong, T. T., Schreck, E., Baeza-Squiban, A., Geret, F., Dumat, C., 2014. Environmental and health impacts of fine and ultrafine metallic particles: assessment of threat scores. *Environmental research*, *133*, 185-194.

Gu Y.-G., Gao Y.-P., Chen F., Huang H.-H., Yu S.-H., Jordan R.W., Jiang S.-J. Risk assessment of heavy metal and pesticide mixtures in aquatic biota using the DGT technique in sediments. *Water Res*. 2022;224:119108.

Gupta, S. P., 2018. Roles of metals in human health. *MOJ Biorg. Org. Chemosphere*, *2*, 221-224.

Hakanson, L., 1980. An ecological risk index for aquatic pollution control. A sedimentological approach. *Water Res*. 14 (8), 975-1001.

Hussain, B., Sultana, T., Sultana, S., Al-Ghanim, K. A., Al-Misned, F., Mahboob, S., 2019. Influence of habitat degradation on the fatty acid profiles of fish, microalgae, and zoobenthos in a river ecosystem. *Process Safety and Environmental Protection*, *123*, 24-32.

Instituto Trata Brasil., 2022. Esgotamento Sanitário inadequado e impactos da saúde da população: Um diagnóstico da situação nos 81 municípios brasileiros com mais de 300 mil habitantes.

Khan T., Muhammad S., Khan B., and Khan H., 2011. Investigating the levels of selected heavy metals in surface water of shah alam river (a tributary of River Kabul, Khyber Pakhtunkhwa), *Journal of Himalayan Earth Sciences*, 44, (2) 71–79.

Koller, M., Saleh, H. M., 2018. Introductory chapter: Introducing heavy metals. *Heavy metals*, *1*, 3-11.

Kusin, F. M., Azani, N. N. M., Hasan, S. N. M. S., Sulong, N. A., 2018. Distribution of heavy metals and metalloid in surface sediments of heavily-mined area for bauxite ore in Pengerang, Malaysia and associated risk assessment. *Catena*, *165*, 454-464.

Lapola, D. M., Pinho, P., Barlow, J., Aragão, L. E., Berenguer, E., Carmenta, R., Walker, W. S., 2023. The drivers and impacts of Amazon forest degradation. *Science*, *379* (6630), eabp8622.

Leveque, T., Capowiez, Y., Schreck, E., Xiong, T., Foucault, Y., Dumat, C., 2014. Earthworm bioturbation influences the phytoavailability of metals released by particles in cultivated soils. *Environmental Pollution*, *191*, 199-206.

Lima, B. D. A. C., Barufatti, A., Cezar, A., 2023. Bioaccumulation of metals and genotoxic effects in females of *Colomesus asellus* collected in an Amazon River estuary, Amapá, Brazil. *Limnetica*, *42* (2), 000-000.

Lima, J. C., Moreira, R. A., Neto, A. J. G., Freitas, E. C., Daam, M. A., Rocha, O., 2023. Toxicity of Binary Metal Mixtures to the Tropical Ostracod *Strandesia Trispinosa*. *Bulletin of Environmental Contamination and Toxicology*, *110* (3), 64.

Lino, A. S., Kasper, D., Guida, Y. S., Thomaz, J. R., Malm, O., 2019. Total and methyl mercury distribution in water, sediment, plankton and fish along the Tapajós River basin in the Brazilian Amazon. *Chemosphere*, *235*, 690-700.

Liu, Y., Liu, G., Yuan, Z., Liu, H., & Lam, P. K., 2018. Heavy metals (As, Hg and V) and stable isotope ratios (δ13C and δ15N) in fish from Yellow River Estuary, China. *Science of the Total Environment*, *613*, 462-471.

Lu, J.; Wang, S.; Li, Y.; F., 2022. Ecological risk assessment of ammonia nitrogen in the rare fish reserve in the upper reaches of the Yangtze River. *Water resources*, 40, 53–56.

Maanan, M., Saddik, M., Maanan, M., Chaibi, M., Assobhei, O., Zourarah, B., 2015. Environmental and ecological risk assessment of heavy metals in sediments of Nador lagoon, Morocco. *Ecological Indicators*, 48, 616-626.

Matos, R., 2005. Espacialidades em rede: população, urbanização e migração no Brasil contemporâneo. *Belo Horizonte: C/Arte*.

Maurya, P. K., Malik, D. S., Yadav, K. K., Kumar, A., Kumar, S., Kamyab, H., 2019. Bioaccumulation and potential sources of heavy metal contamination in fish species in River Ganga basin: Possible human health risks evaluation. *Toxicology reports*, *6*, 472-481.

Mebane, C. A., T. S. Schmidt, J. L. Miller., Balistrieri. L. S., 2020. Bioaccumulation and toxicity of cadmium, copper, nickel, and zinc and their mixtures to aquatic insect communities. *Environmental Toxicology and Chemistry*, 39: 812–833.

Mishra, S., Bharagava, R. N., More, N., Yadav, A., Zainith, S., Mani, S., Chowdhary, P., 2019. Heavy metal contamination: an alarming threat to environment and human health. *Environmental biotechnology: For sustainable future*, 103-125.

Mongeon, P., & Paul-Hus, A., 2016. The journal coverage of Web of Science and Scopus: a comparative analysis. *Scientometrics*, 106 (1), 213-228.

Mount, D.I., Norberg, T.J., 1984. A seven-day life cycle cladoceran toxicity test. *Environmental Toxicology and Chemistry*, 3, 425-434.

Moulatlet, G. M., Yacelga, N., Rico, A., Mora, A., Hauser-Davis, R. A., Cabrera, M., Capparelli, M. V. (2023). A systematic review on metal contamination due to mining activities in the Amazon basin and associated environmental hazards. *Chemosphere*, 139700.

Nascimento, T. S. R., do Nascimento Monte, C., Correa, E. S. (2021). Qualidade de água em áreas influenciadas por uma estação de tratamento de esgoto na Amazônia. *Revista Ibero-Americana de Ciências Ambientais*, *12*(9), 146-160.

Oliveira. G. M. T. S. D., Oliveira. E. S. D., Santos. M. D. L. S., Melo. N. F. A. C. D. Krag. M. N., 2018. Concentrações de metais pesados nos sedimentos do lago Água Preta (Pará. Brasil). *Engenharia Sanitaria e Ambiental*. *23*, 599-605.

Real, M., Munoz, I., Guasch, H., Navarro, E., Sabater, S., 2003. The effect of copper exposure on a simple aquatic food chain. *Aquatic toxicology*, *63* (3), 283-291.

Rebêlo, A. G. D. M., Monteiro, M. T. F., Ferreira, S. J. F., Ríos-Villamizar, E. A., Quesada, C. A. N., Duvoisin Junior, S., 2020. Reference values of heavy metal concentration in soils in central Amazonia. *New* Chemistry, *43*, 534-539.

Ribeiro, D. R. G., Faccin, H., Dal Molin, T. R., de Carvalho, L. M., Amado, L. L. 2017. Metal and metalloid distribution in different environmental compartments of the middle Xingu River in the Amazon, Brazil. *Science of the Total Environment*, *605*, 66-74.

Rico, A., de Oliveira, R., de Souza Nunes, G. S., Rizzi, C., Villa, S., Vizioli, B. D. C., Waichman, A. V., 2022. Ecological risk assessment of pesticides in urban streams of the Brazilian Amazon. *Chemosphere*, *291*, 132821.

Rico, A., de Oliveira, R., de Souza Nunes, G. S., Rizzi, C., Villa, S., López-Heras, I., Waichman, A. V. (2021). Pharmaceuticals and other urban contaminants threaten Amazonian freshwater ecosystems. *Environment International*, *155*, 106702.

Rosabal, M., Pierron, F., Couture, P., Baudrimont, M., Hare, L., Campbell, P. G., 2015. Subcellular partitioning of non-essential trace metals (Ag, As, Cd, Ni, Pb, and Tl) in livers of American (Anguilla rostrata) and European (Anguilla anguilla) yellow eels. *Aquatic Toxicology*, *160*, 128-141.

Rocha, O., Neto, A. J. G., dos Santos Lima, J. C., Freitas, E. C., Miguel, M., da Silva Mansano, A., Daam, M. A., 2018. Sensitivities of three tropical indigenous freshwater invertebrates to single and mixture exposures of diuron and carbofuran and their commercial formulations. *Ecotoxicology*, *27*, 834-844.

Sadeq, S. A., Beckerman, A. P., 2019. The chronic effects of copper and cadmium on life history traits across Cladocera species: a meta-analysis. *Archives of environmental contamination and toxicology*, *76*, 1-16.

Santana. G. P., Barroncas. P. D. S. R., 2007. Estudo de metais pesados (Co. Cu. Fe. Cr. Ni. Mn. Pb e Zn) na Bacia do Tarumã-Açu Manaus (AM). *Acta Amazonica*. *37*. 111-118.

Sheppard, S., Long, J., Sanipelli, B., Sohlenius, G., 2009. *Solid/liquid partition coefficients (Kd) for selected soils and sediments at Forsmark and Laxemar-Simpevarp* (No. SKB-R--09-27).

Silva, M. H. L., de Castro, A. C. L., da Silva, I. S., Cabral, P. F. P., de Jesus Azevedo, J. W., Soares, L. S., Nunes, J. L. S., 2023. Determination of metals in estuarine fishes in a metropolitan region of the coastal zone of the Brazilian Amazon. *Marine Pollution Bulletin*, *186*, 114477.

Singh, K. P., Mohan, D., Singh, V. K., Malik, A., 2005. Studies on distribution and fractionation of heavy metals in Gomti river sediments—a tributary of the Ganges, India. *Journal of hydrology*, *312* (1-4), 14-27.

Sotero-Santos, R. B., Rocha, O., Povinelli, J., 2007. Toxicity of ferric chloride sludge to aquatic organisms. *Chemosphere*, *68* (4), 628-636.

Souza, C. A. S., 2000. Urbanização na Amazônia. Belém do Pará: Unama.

Suedel, B. C., Rodgers, Jr. J. H., Deaver, E., 1997. Experimental factors that may affect toxicity of cadmium to freshwater organisms. *Archives of Environmental Contamination and Toxicology, 33*, 188-193.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., Sutton, D. J., 2012. Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology: volume 3: environmental toxicology*, 133-164.

Thorley, J., Schwarz C., 2018. ssdtools: An R package to fit Species Sensitivity Distributions. *Journal of Open Source Software*, 3 (31), 1082.

Tonani, K. A. D. A., 2008. *Identificação e quantificação de metais pesados, parasitas e bactérias em esgoto bruto e tratado da Estação de Tratamento de Esgoto de Ribeirão Preto-SP* (Doctoral dissertation, Universidade de São Paulo).

USEPA., 2005. Partition coefficients for metals in surface water, soil, and waste. *Rep. EPA/600/R-05*, *74*.

Valdés, J., Guiñez, M., Castillo, A., Vega, S. E., 2014. Cu, Pb, and Zn content in sediments and benthic organisms from San Jorge Bay (northern Chile): Accumulation and biotransference in subtidal coastal systems. *Ciencias Marinas*, *40* (1), 45-58.

Van Vlaardingen, P., Traas, T., Wintersen, A., Aldenberg, T., 2004. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. *National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands*.

Vatandoost, M., Naghipour, D., Omidi, S., Ashrafi, S. D., 2018. Survey and mapping of heavy metals in groundwater resources around the region of the Anzali International Wetland; a dataset. *Data in brief*, *18*, 463-469.

Viana, L. F., Kummrow, F., Cardoso, C. A. L., de Lima, N. A., Solórzano, J. C. J., do A. Crispim, B., Florentino, A. C. 2021. High concentrations of metals in the waters from Araguari River lower section (Amazon biome): Relationship with land use and cover, ecotoxicological effects and risks to aquatic biota. *Chemosphere*, 285, 131451.

Yan, F., Li, N., Yang, Z., & Qian, B., 2022. Ecological Risk Evaluation of Baihetan Dam Based on Fuzzy Hazard Quotient Model. *Water*, *14* (17), 2694.

Zafarzadeh, A., Bay, A., Fakhri, Y., Keramati, H., Hosseini Pouya, R., 2018. Heavy metal (Pb, Cu, Zn, and Cd) concentrations in the water and muscle of common carp (Cyprinus carpio) fish and associated non-carcinogenic risk assessment: Alagol wetland in the Golestan, Iran. *Toxin reviews*, *37* (2), 154-160.

Zhou, Q., Yang, N., Li, Y., Ren, B., Ding, X., Bian, H., Yao, X., 2020. Total concentrations and sources of heavy metal pollution in global river and lake water bodies from 1972 to 2017. *Global Ecology and Conservation*, *22*, e00925.

Chapter 2 - On a new species of ostracod from the Brazilian Amazon and its potential for experimental studies in laboratory culture

Submitted in Limnologica (Manuscript Number: LIMNO-D-24-00005)

On a new species of ostracod from the Brazilian Amazon and its potential for experimental studies in laboratory culture

Abstract

Ostracods are taxonomically and ecologically diverse small benthic crustaceans that have recently gained prominence in laboratory studies and environmental impact assessment. In this context, the present study aims to assess the applicability of a new freshwater ostracod for experimental studies in the laboratory, and we provide the formal description of *Strandesia rondoniensis* **n. sp**. The original specimens for setting the laboratory cultures originated from the Natural Park of Porto Velho, in the Amazon region (Rondônia State, Brazil). The growth and reproductive rates of eleven adult individuals of *S. rondoniensis* **n. sp**. were analyzed. The results showed a high morphological resemblance with *Neostrandesia striata* and *Bradleytriebella lineata*, even though the new species belongs to *Strandesia*, indicating evolutionary convergence. The life cycle analysis showed that individuals of *S. rondoniensis* **n. sp**. have fast growth and high reproductive rates, which favour their use in laboratory studies. Besides contributing to the knowledge about ostracods in the Amazon region, which has been poorly studied, the life cycle experiment characterizations provided here should promote the use of this new species as a model organism for laboratory studies.

Key words: aquatic ecosystem, cypricercinae, evolutive convergence, life cycle, *Strandesia*

1. Introduction

Ostracods are small benthic crustaceans and constitute one of the most diverse taxonomic groups in aquatic ecosystems (Horne et al. 2002; Coviaga et al. 2015). They can occur in marine or freshwater environments and are classified into two subclasses: Myodocopa and Podocopa (Horne et al. 2002). While the Myodocopa are exclusively marine, the Podocopa have three superfamilies (Cypridoidea, Cytheroidea and Darwinuloidea) with freshwater representatives (Higuti, Roche & Martens, 2016). The family Cyprididae (Cypridoidea) currently comprises 43.2% of the global freshwater ostracod diversity, which is the most speciose in this environment (Meisch, Smith & Martens, 2019). Within this family, the subfamily Cypricercinae is also highly diverse, whereby 172 species are distributed in 12 genera worldwide (Ferreira, Higuti & Martens, 2019). Of these 12 genera, the first to be described and the most diverse is *Strandesia*, described by Stuhlmann in 1888 and comprising 97 species (Meisch, Smith & Martens, 2019). The subfamily Cypricercinae and the genus *Strandesia* are, however, characterized not only by their high diversity but also by their long history of taxonomic unclearness. Some factors can explain this, such as the lack of consistent morphological characteristics to divide the different tribes and genera, the mosaic pattern of several characters traditionally used to classify such tribes and genera, or the description of dozens of species as belonging to the genus *Strandesia* despite the fact of being significantly different from the type species *Strandesia mercatorum* (Vavra, 1895) (Savatenalinton & Martens, 2009c, 2010; Ferreira, Higuti & Martens, 2019).

Another factor contributing to taxonomic inconsistency is the existence of evolutive convergent clusters of species, a frequent but poorly explored phenomenon within Ostracoda (Ferreira, Higuti & Martens, 2019). One such example, reported by Ferreira, Higuti & Martens (2019), is the evolutive convergence of *N. striata* from Brazil with the widely distributed species *Bradleytriebella lineata* (Victor & Fernando, 1981).

Cultivation of aquatic organisms under controlled conditions, allows the investigation of deeper and finer information on the development of individuals, such as body growth, reproduction and longevity (Silva et al. 2014; Silva et al. 2023). Data of this nature often provide relevant information for species characterization and usage in ecotoxicological studies (Freitas & Rocha, 2006; Mansano et al., 2018; Lima et al., 2023). Concerning this matter, Sevilla, Nakajima and Kasuga (2014), Niyommaneerat et al. (2017), and Miriani et al. (2022) already used the standardized species (ISO 14371, 2012)

Heterocypris incongruens in tests with different types of environmental pollutants while Khangarot & Das (2009), Shuhaimi-Othman et al. (2011), Lima et al. (2019, 2023) and Chen et al. (2022) evaluated the sensitivity of different species of ostracods to several metals.

Here, we assess the applicability of a new freshwater ostracod for experimental studies in the laboratory. We provide the formal taxonomic description of *S. rondoniensis* **n. sp**., and the characterization of its life cycle in laboratory cultures. The taxonomic description contributes to the knowledge about ostracods in the Amazon region, which has been poorly studied. Morphological results are discussed regarding the convergent nature of the new species with *Neostrandesia striata* and *Bradleytriebella. lineata*. Finally, the life cycle aspects of cultured specimens provided here should promote the use of this new species as a model organism for laboratory studies.

2. Material and Methods

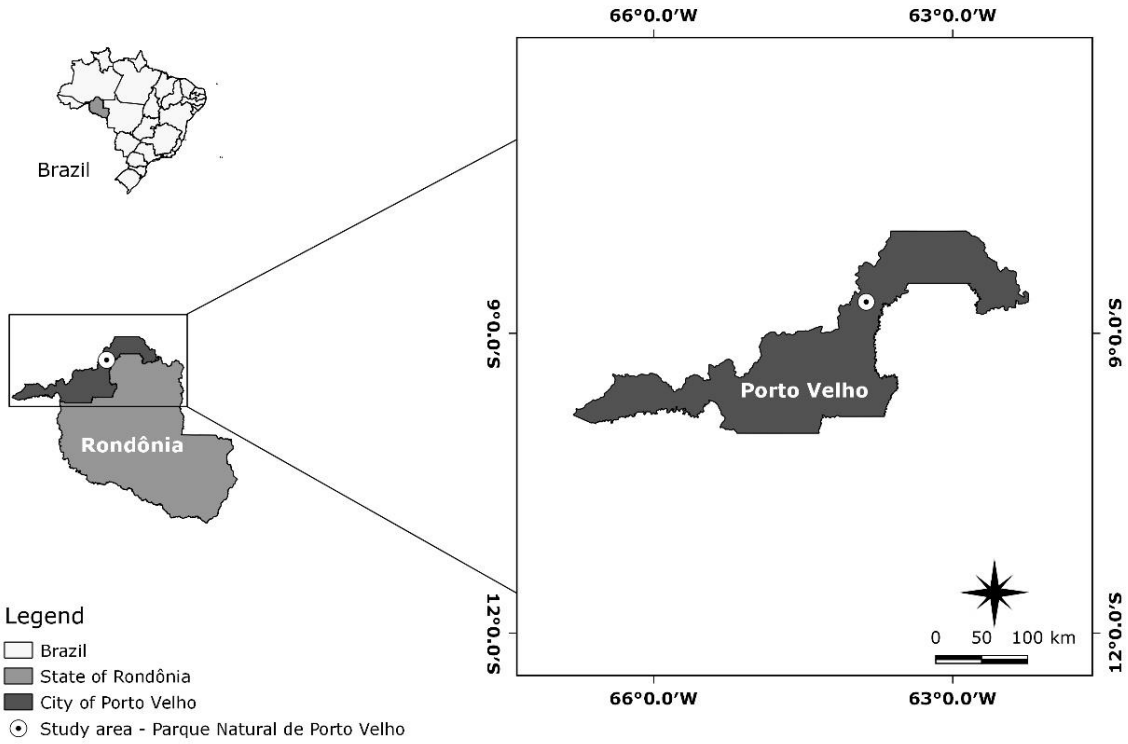
2.1 Ostracod Sampling and Cultivation

The sample collection occurred in the natural park of Porto Velho (Fig. 1), Rondônia state, Brazil, which belongs to the Amazonian biome. A hand net was used to concentrate material from the water near the river margin and macrophyte banks. Samples were transferred to plastic bottle and taken to the Limnology and Aquatic Ecotoxicology Laboratory at the Universidade Federal de São Carlos (UFSCar). Environmental variables were measured for the following parameters: water temperature (Cº), electrical conductivity (µS cm^{-1}), dissolved oxygen (mg L^{-1}) and pH. The physical-chemical variables of the collection points were measured in triplicate using the AKSO AK59 Probes (for pH, temperature and electrical conductivity) and AKSO DO Eco for dissolved oxygen readings. Sampling occurred with the authorization of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) under the number 67974-1.

At the laboratory, ostracods were sorted and cultivated in an Ethiktechnology® BOD model 411FPD incubator in a 5 L beaker with 500 g of calcined sediment (550 ºC/5 h) and 4 L of reconstituted water. Reconstituted water was prepared following a protocol for tropical cladocerans (ABNT, 2016), pH 7–7.6, hardness 40–48 mg L^{-1} of $CaCO_3$, and electrical conductivity of 160 µS cm^{-1}. The diet consisted of 20 mL of Tetramin® fish food solution (2 g per liter of distilled water) and 20 mL of *Chlorella sorokiniana*

suspension (≈ 10^8 cells L^{-1}). Cultures were kept under controlled photoperiod (12 h light: 12 h dark) and temperature (25 ± 1 °C). Adults from these cultures were separated and preserved in 70% alcohol. Taxonomic analysis ensued at the Entomology Laboratory and Marine Invertebrates Laboratory Paulo Young, Universidade Federal da Paraíba (UFPB), and at the Laboratory of Micropaleontology of the University of Brasília (UnB).

Figure. 1 - Location of the natural park of Porto Velho - RO.



2.2 Taxonomy

For taxonomic classification, specimens were dissected under a stereomicroscope Leica EZ4. Hard parts (carapace) were stored dry in micropaleontological slides. Soft parts (appendages) were mounted on glass slides with glycerin 87% as a mounting medium, covered with lama coverslip, and sealed with clear nail polish. Drawing of the appendages was done under the compound microscopes Olympus BX41 and Zeiss Axioskop, at Laboratório de Invertebrados Marinhos Paulo Yong (UFPB) and the Micropaleontology Laboratory (UnB), respectively. Carapaces were imaged with a scanning electron microscope JEOL NeoScope JCM-5000 at the Micropaleontology

Laboratory (UnB). The final art of appendage drawings and carapace photomicrographs were produced with Adobe Illustrator CS6 and Photoshop CS6, respectively.

The type material is deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP). The higher taxonomy follows Horne et al. (2002), Savatenalinton & Martens (2009c) and Meisch et al. (2019). The literature used for morphological comparisons is detailed in Table 1.

Table 1 - Source of information used for the taxonomic comparison between the three evolutive convergent species: *N. striata*, *B. lineata* and *S. rondoniensis* **n**. **sp.**

Species	Source of information
Neostrandesia striata	Ferreira, Higuti & Martens (2019) original description
Bradleytriebella lineata	Victor & Fernando (1981) original description (as *Strandesia lineata*)
	Savatenalinton & Martens (2010) abbreviated redescription
	Ferreira, Higuti & Martens (2019) fully redescription
	Martens (1984) (as *Paracypretta amati*, latter synonymized)
	Okubo (2004) (as *Strandesia biwaensis*, latter synonymized)
Strandesia rondoniensi **n. sp.**	Here described

Abbreviations used in text and figures

Valves

DV = dorsal view

H = height

iv = internal view

L = length

RLV = right lateral view

VV = ventral view

W = width

Appendages

A1 = antennula

A2 = antenna

Ex = exopodite

Mx = maxillula

R = Rome organ

T1 = first thoracic limb

y3 = antenna terminal segment aesthethasc

Y = antenna aesthethasc

ya = antennula aesthethasc

2.3 Life cycle and post-embryonic development

The life cycles of eleven specimens were studied. Each organism was observed daily in an excavated glass under a stereomicroscope at 50x magnification and handled with broad Pasteur pipettes. The newborns (1 day old) were placed in non-toxic plastic cups containing 50mL of culture medium and cultivated under the same conditions as the stock culture. The specimen size was measured daily from birth until death. The initial curvature was fitted with the data with a Ford-Walford transformation to define the initial parameter L (Sparre & Venema, 1998):

Eq. 1 - $Lt = L\infty [1 – e – K (t – t0)]$

Where Lt = fulfillment at time t (mm), L = maximum length (mm), K = growth rate constant (d-1), e = base of natural logarithms, t0 = theoretical time before birth when the model extrapolates to zero length. The age at first reproduction consists of the time elapsed between the birth of the ostracod newborn and its first progeny. Determination of the unit considered the number of newborn produced per day per individual. New hatchlings were removed at the time of observation. Lifetime (length of life cycle) refers to the total time elapsed between the birth and death of the organism.

3. Results

3.1 Taxonomy

Class Ostracoda Latreille, 1802

Subclass Podocopa Sars, 1866

Order Podocopida Sars, 1866

Suborder Cypridocopina Baird, 1845

Superfamily Cypridoidea Baird, 1845

Family Cyprididae Baird, 1845

Subfamily Cypricercinae McKenzie, 1971

Tribe Cypricercini McKenzie, 1971

Genus *Strandesia* Stuhlmann, 1888

Type species *Strandesia mercatorum* (Vávra, 1895)

Diagnosis. See Savatenalinton & Martens (2009b).

***Strandesia rondoniensis* n. sp. Pereira, Gomes and Pinto**

Fig. 2–5

Diagnosis. Small-sized (length of female = 657.32 µm) species with tumid carapace (height/length ratio = 0.7). Surface with normal pore canals, numerous setae and subtle ornamentation of reticulated pattern. Left valve overlapping right one. In dorsal and ventral views, posterior contour symmetric, acuminate; anterior contour strongly asymmetric. Margins sinuous in dorsal and ventral views. In right lateral view, carapace sub-triangular, greatest height at mid-length; left valve overlaps right one on all margins except the postero-dorsal one; posterior margin acuminate, anterior margin narrowly rounded, ventral margin sinuous. Internally, a groove and one inner list present on left valve anterior region; selvage present on right valve ventral, posterior and dorsal margins. Wouters and Rome organs present on antennula; five setae on the 6th segment of antennula; short aesthethasc (Y) on antenna; 6+1 setae pattern on the maxillular palp; two sideways-directed bristles on the maxillula; long b-seta on the first thoracic limb; d-seta present on first thoracic limb.

Etymology

The specific epithet "*rondoniensis*" refers to the State of Rondônia, Brazil, where the species was originally collected (see type locality below).

Type Material. Holotype: a dissected female (JP125ab) with valves dried and coated for scanning electron microscopy stored in a micropaleontological slide and appendages mounted in a sealed slide with glycerin.

Paratypes: seven females (JP126ab, JP127ab, JP128ab, JP129ab, JP130ab, JP160a, JP272ab) dissected and stored like the holotype; two females (JP216, JP217) dried and coated for scanning electron microscopy stored in micropaleontological slides; numerous females (MZUSP: will be provided soon) kept whole in a vial with 70% alcohol.

Type Locality. River margin from Ecological Park of Porto Velho, Rondônia, Brazil. Water associated to macrophytes. Geographic coordinates: 8º41'13.26" S, 63º52'10.06" W. Material collected in August, 2018 by Gomes, D. F.

Description of the female

Carapace (Fig. 2A–L). Small-sized (L = 657.32 µm), greatest width at mid-length. Surface with normal pore canals, numerous setae, subtle ornamentation: reticulated pattern. In dorsal and ventral views (Fig. 2A, B), carapace tumid (H/L ratio = 0.7); posterior contour symmetric, acuminate; anterior contour asymmetric: left valve greatly longer than right valve. In dorsal view (Fig. 2A), dorsal ridge not tightly enclosed in the anterior and posterior regions. In the ventral view (Fig. 2B), central portion flat; ventral ridge not tightly enclosed in the anterior region, one flap in the ventral ridge, setae line along major and minor ventral ridges. In the right lateral view (Fig. 2D–F), carapace sub-triangular, greatest height at mid-length; left valve overlaps right one on all margins except postero-dorsal one; posterior margin acuminate, anterior margin narrowly rounded, ventral margin sinuous.

Left valve internal view (Fig. 2J–K). Sub-triangular, posterior margin acuminate, anterior margin narrowly rounded. Groove from antero-dorsal to posterior margin, one inner list on the anterior calcified inner lamella. Calcified inner lamella and vestibule narrow in the anterior and posterior regions. Selvage absent.

Right valve internal view (Fig. 2G–I). Sub-triangular, posterior margin acuminate, anterior margin broadly rounded. No groove or inner list on anterior calcified inner lamella. Calcified inner lamella and vestibule narrow in the anterior region, almost absent in the posterior one. Selvage absent on anterior margin, weakly-inwardly on ventral, posterior and dorsal margins.

Antennula (Fig. 3B–G). Seven-segmented. First segment (Fig. 3B) broad; long Wouters organ dorsally; one short (reaching tip of segment) dorso sub-apical seta, two unequal long, ventro sub-apical setae. Second segment (Fig. 3B) flattened (length ~2/3 of width); long Rome organ ventro-apically; short seta dorso-apically. Third segment (Fig. 3C) rectangular: length two times larger than width; one slender dorso-apical seta reaching beyond fourth segment apex, one ventro-apical seta reaching about fourth segment length. Fourth segment (Fig. 3D) square-shaped; two greatly long dorso-apical setae (reaching beyond seventh segment apex), two short, ventro-apical setae: one half-length the other. Fifth segment (Fig. 3E) square-shaped; two greatly long dorso-apical setae (>2 times longer than medial seta length), one long, slender medial-apical seta, one short ventro-apical seta. Sixth segment (Fig. 3F) short, rectangular; two short, thinner dorso-apical setae: one half-length the other, two greatly long medial-apical setae, one slender, ventro-apical seta reaching medial setae mid-length. Seventh segment (terminal) (Fig. 3G) thin, rectangular (>2 times longer than wider); three apical setae: two dorsal sub-equal long setae, one ventral short, slender seta; plus a dorsal aesthethasc (Ya) longer than the shortest seta.

Antenna (Fig. 3A). Protopodite two-segmented. Coxa rounded; one long, medial seta, two long, ventro-apical setae. Basis long and curved; exopodite a long, slender seta (not reaching first endopodal segment apex) plus two-minute setae; one slender, long seta (reaching second endopodal segment apex), ventrally. First endopodal segment the longest; five greatly long natatory setae (reaching beyond distal claws) plus one short seta (not reaching second endopodal mid-length), dorso-apically; short aesthethasc (Y) with chemosensorial section ~50% of its length, ventrally; one long seta (reaching beyond second endopodal segment mid-length) plus one-minute vestigial seta, ventro-apically. Second endopodal segment long, partially sub-divided at mid-length; at mid-length: two sub-equal setae, dorsally, four setae (t1–t4) ventrally; apically: three serrated claws (G1–G3), three equal-length long, slender setae (z1–z3) plus an aesthethasc (y2) reaching terminal segment apex; Third endopodal segment (terminal) the shortest; two serrated claws (GM and Gm, the former stouter and longer) apically, one short g seta medial-apically, a short aesthethasc (y3) with slender accompanying seta, ventro-apically.

Mandible (Fig. 3H, 4A, B). Coxa (Fig. 3H) arched and strong; apically a strong row of six tricuspid teeth interleaved by three setae; three ventro-apically setae: two equal-length, one longer; dorsally, one sub-apical short seta. Protopodite (Fig. 4A, B) four-segment. First segment long; two large setae (s1, s2) filled with numerous

pseudochaetae plus one long seta, ventral-apically. Second segment flattened: width five times the length; two setae dorso-apically: one long (reaching beyond fourth segment apex), one short (reaching third segment mid-length); five setae ventro-apically: three equal-length, long setae (reaching beyond fourth segment apex), one shorter seta (reaching fourth segment apex) plus a β-seta with globular basis and pointed apex, filled with numerous pseudochaetae. Third segment rectangular-shaped; dorsally: three equal-length slender setae sub-apically, a γ-seta apically, three equal-length slender setae medial-apically; ventrally: one long seta apically. Fourth segment (terminal) (Fig. 4B) the shortest; three slender claws plus three thin setae.

Maxillula (Fig. 4C). Protopodite two-segmented. First segment the longest; five sub-equal length setae, dorso-apically plus one seta sub-apically; one long seta (reaching second segment apex), ventrally. Second segment small; three slender claws plus three slender setae, apically. Third endite with about seven slender setae dorso-apically: six long, one shorter; two claws medial-apically; one long ventro-apical seta plus one long ventro-basal seta. Second endite with ventro sub-apical short seta plus about nine approximately equal-length slender setae, apically. First endite with about 9 approximately equal-length short setae, apically plus two sub-equal sideways-directed bristles. Respiratory plate a row of setae filled with pseudochaetae.

First thoracic limb (Fig. 5A). Protopodite long; two a-setae: one shorter than the other; one long b-seta; one short sub-apical d-seta; about 14 unequal hirsute setae, apically. Endopodite delicate; three unequal slender apical setae.

Second thoracic limb (Fig. 5B). First segment filled with trabeculae, one long, slender seta (d1), dorsally. Second segment filled with trabeculae, one slender seta (d2) reaching half the length of d1, dorsally. Third segment the longest; one dorso-apical long seta (e) (reaching beyond fourth segment apex). Fourth segment rectangular-shaped; one dorso-apical slender, long seta (f) (reaching beyond sixth segment apex). Fifth segment rectangular-shaped; one dorso-apical short seta (g) (reaching slightly beyond sixth segment apex). Sixth segment (terminal) the smallest; two short setae: one dorsally (h1), one ventrally (h3), the latter shorter, plus a distal claw (h2) serrated in less than half of its length.

Third thoracic limb (Fig. 5C). A cleaning limb. First segment long, curved; three long, slim setae: two dorsally, one ventrally. Second segment elongated (length >2 times the width); one long dorso-apical slim seta (e) (reaching third segment half-length). Third segment elongated, curved; one short (not reaching segment apex), slim, dorso-medial

seta (g); Fourth segment (terminal) the shortest; an apical pincer (h2) plus a long ventro sub-apical reflexed seta (h3) and a vestigial seta (h1).

Caudal ramus (Fig. 5D). Slender, ventral margin slightly serrated. Distal and proximal claws slim, slightly serrated: distal claw long, proximal claw shorter (reaching distal claw half-length). Distal seta slim, reaching about distal claw half-length. Proximal seta slim, short (not reaching ramus apex), proximal seta insertion far from proximal claw insertion.

Caudal ramus attachment (Fig. 5D). Long, slim. Triebel's loop on weakly-developed dorsal branch, ventral branch long and thin.

Measurements

Female. Length = 657.32 µm. Width = 434.15 µm. Height = 439.63 µm.

Differential diagnosis. *S. rondoniensis* **n**. **sp.** resembles two other species from related Cypricercinae genera: *B. lineata* and *N. striata*. Concerning the carapace, *S. rondoniensis* **n**. **sp**. can be differentiated from the other two by the ornamentation pattern, which, although similar, is reticulate in *S. rondoniensis* **n**. **sp.** instead of striate as in the other two; the dorsal ridge (DV) and the ventral ridge (VV) weakly instead of tightly enclosed as occurs is *B. Lineata* and *N. striata*; and, in right lateral view, the greatest height situated at mid-length, the posterior contour acuminated and the anterior contour narrowly rounded instead of the pattern of greatest height situated anteriorly and posterior/anterior contour broadly rounded exhibited by both *B. lineata* and *N. striata*. Additionally, *S. rondoniensis* **n**. **sp.** can be differentiated from *N. striata* also by the presence of an inner list on left valve anterior calcified lamella (absent in *N. striata*) and the absence of crenulation on the right valve anterior margin (present in *N. striata*). Visible differences of carapace are listed in Table 2. Concerning the appendages, *S. rondoniensis* **n**. **sp.** can be differentiated from *B. lineata* by the short (instead of long) aesthetasc on A2, the presence of a d-seta on T1, the presence of two (instead of one) sideways-directed bristles on Mx and five (instead of three) setae on the 6th segment of A1. Finally, *S. rondoniensis* **n**. **sp.** can be differentiated from *N. striata* by the presence of Wouters and Rome organs on A1, the long (instead of giant) b-seta on T1 and the pattern of 6+1 setae on Mx instead of 4+1 as found in *N. striata*. The main differences among these three species concerning appendages are summarized in Table 3 and fully detailed in the Discussion Section.

Distribution

The species is currently known only from the type locality.

Table 2 - Comparison between the three evolutive convergent species *Neostrandesia striata*, *B. lineata* and *Strandesia rondoniensis* **n. sp.** concerning carapace characteristics.

Species/ Character	Ornamentation pattern	Dorsal ridge (DV)	Ventral ridge (VV)	Greatest height position (RLV)	Ventral margin (RLV)	Anterior contour (DV/VV) asymmetry	Posterior contour (RLV)	Selvage presence (iv)	Anterior inner list (iv)
Neostrandesia striata	striated	tightly enclosed	tightly enclosed	anteriorly	rounded	subtle	rounded	all margins	absent
Bradleytriebella lineata	striated	tightly enclosed	tightly enclosed	anteriorly	sinuous	strong	rounded	dorsal, posterior	1
Strandesia rondoniensi **n. sp.**	reticulated	not tightly enclosed	not tightly enclosed	mid-length	sinuous	strong	acuminated	dorsal, posterior, ventral	1

Table 3 - Comparison among the three evolutive convergent species *N. striata*, *B. lineata* and *S. rondoniensis* **n. sp.** for nine main taxonomic diagnostic characters, concerning appendages.

Species/ Character	A1 Wouters organ	A1 Rome organ	A1, 6th segment, setae	A2 aesthetasc Y	Mx, 1st segment palp, setae	Mx, 2nd segment palp, setae	Mx, sideways-directed bristles	T1 b-seta	T1 d-seta
Neostrandesia striata	absent?	absent?	?	short	4+1	1+3	2	giant	present
Bradleytriebella lineata	present	present	3?	long	6+1	3+3	1	long	absent
Strandesia rondoniensis **n. sp.**	present	present	5	short	6+1	3+3	2	long	present

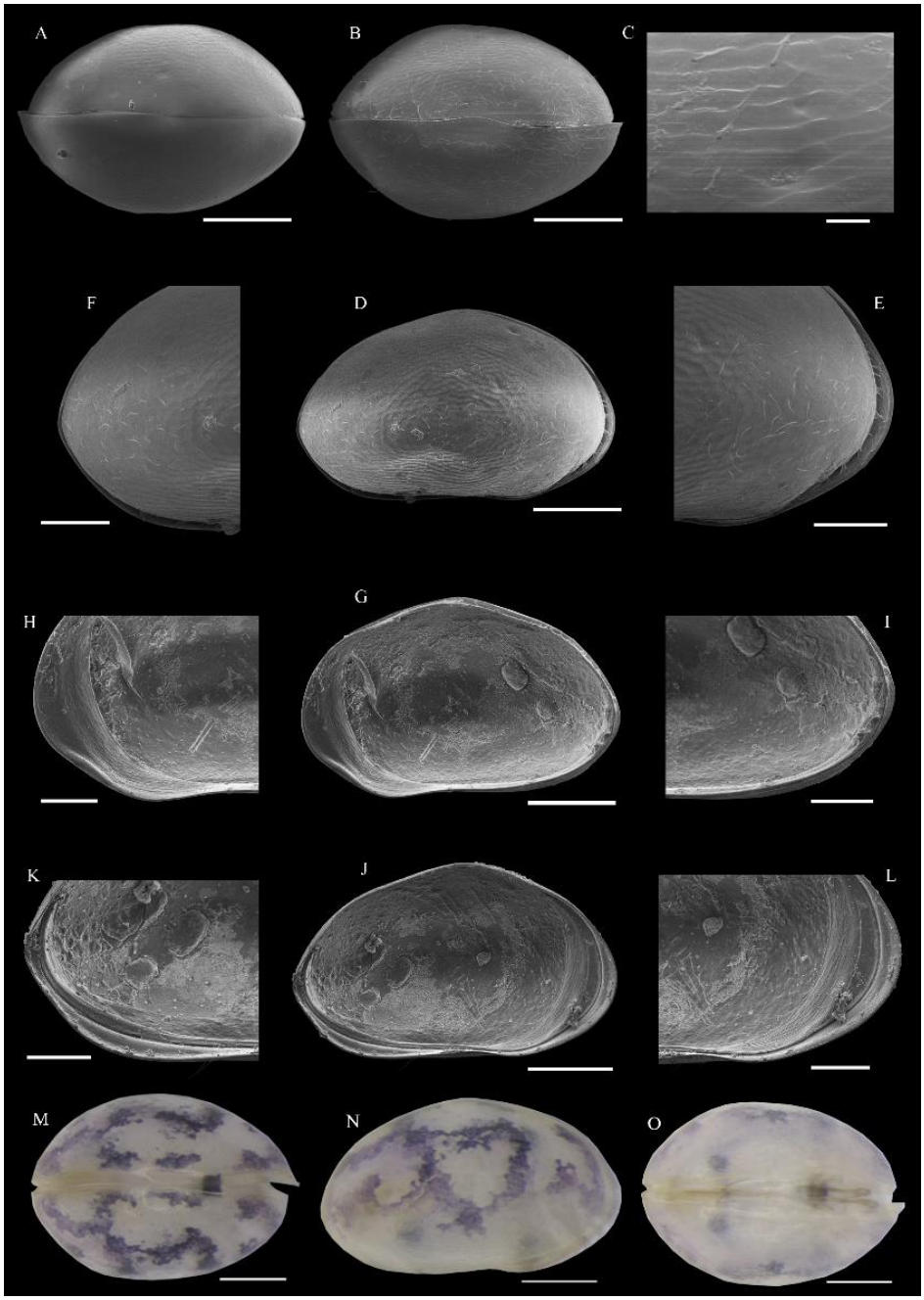


Fig. 2 - *Strandesia rondoniensis* **n**. **sp.**, female. A- dorsal view (JP217); B- ventral view (JP216); C- dorsal view, detail showing the ornamentation pattern (JP217); D- right lateral view (JP216); E- right lateral view, detail of anterior region (JP216); F- right lateral view, detail posterior (JP216); G- right valve, internal view (JP125b); H- right valve, internal view, detail of anterior region (JP125b); I- right valve, internal view, detail of posterior region (JP125b); J- left valve, internal view (JP125b); K- left valve, internal view, detail of posterior region (JP125b); L- left valve, internal view, detail of anterior region (JP125b); M- dorsal view; N- right lateral view; O- ventral view. Scale bars: A, B, D, G, J– 200µm, C– 20µm, E, F, H, I, K, L– 100µm, M–O- 0.2mm.

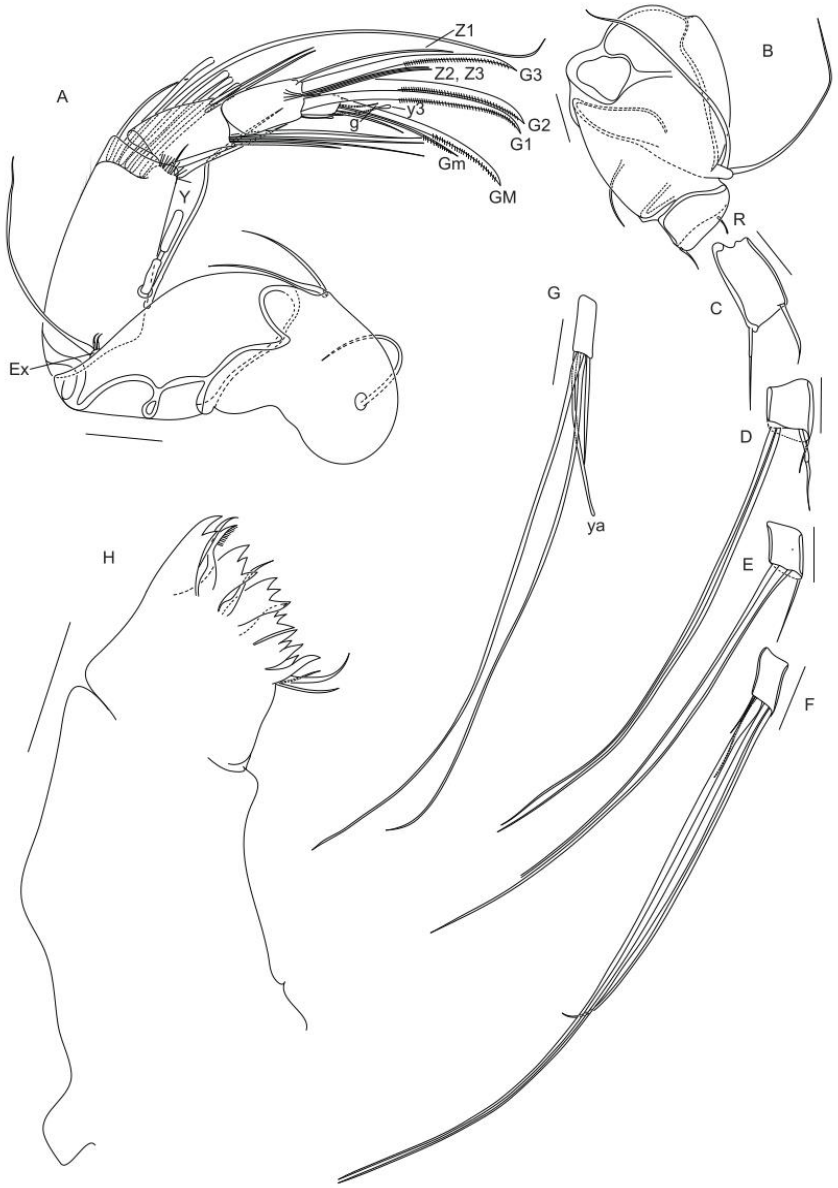


Fig. 3 - *Strandesia rondoniensis* **n. sp.**, female. A- antenna (JP125a); B- antennula, first and second appendages (JP130a); C- antennula, third appendage (JP130a); D- antennula, fourth appendage (JP130a); E- antennula, fifth appendage (JP130a); F- antennula, sixth appendage (JP130a); G- antennula, seventh appendage (JP130a); H- mandible, coxa (JP126a). Scale bars: A- 0.05mm, B–H- 0.01mm.

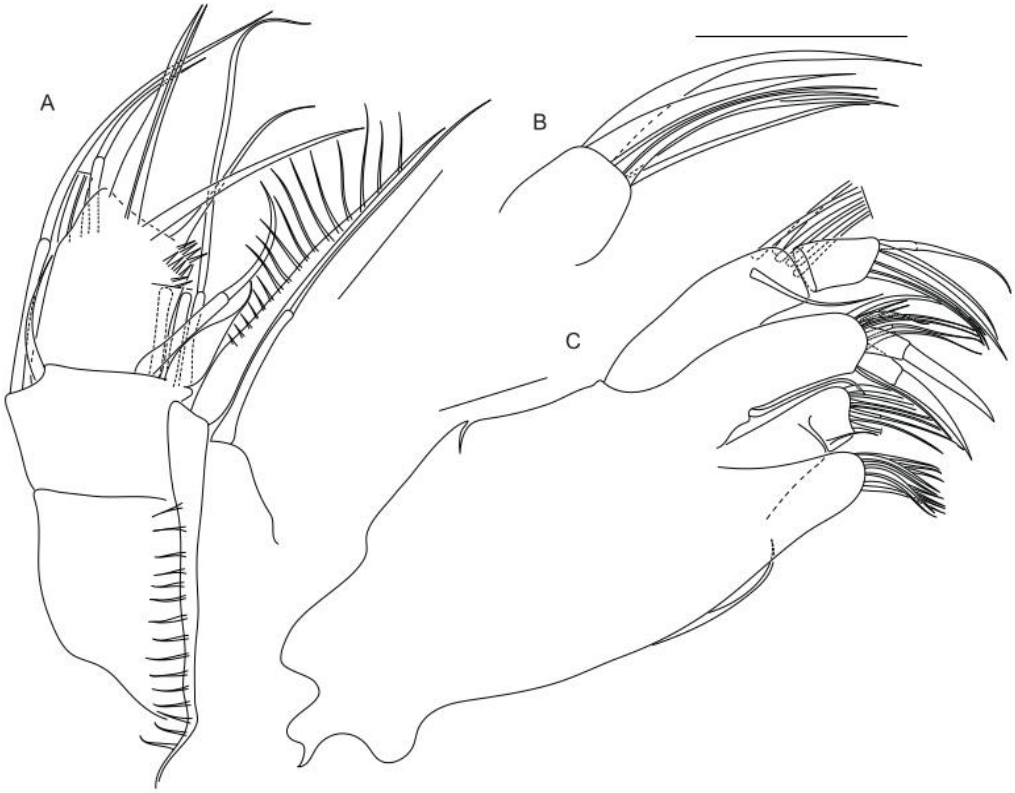


Fig. 4 - *Strandesia rondoniensis* **n. sp.**, female. A- mandible, palp (JP126a); B- mandible, palp, fourth (terminal) segment (JP126a); C- maxillula, palp (JP160a). Scale bars: A–C- 0.01mm.

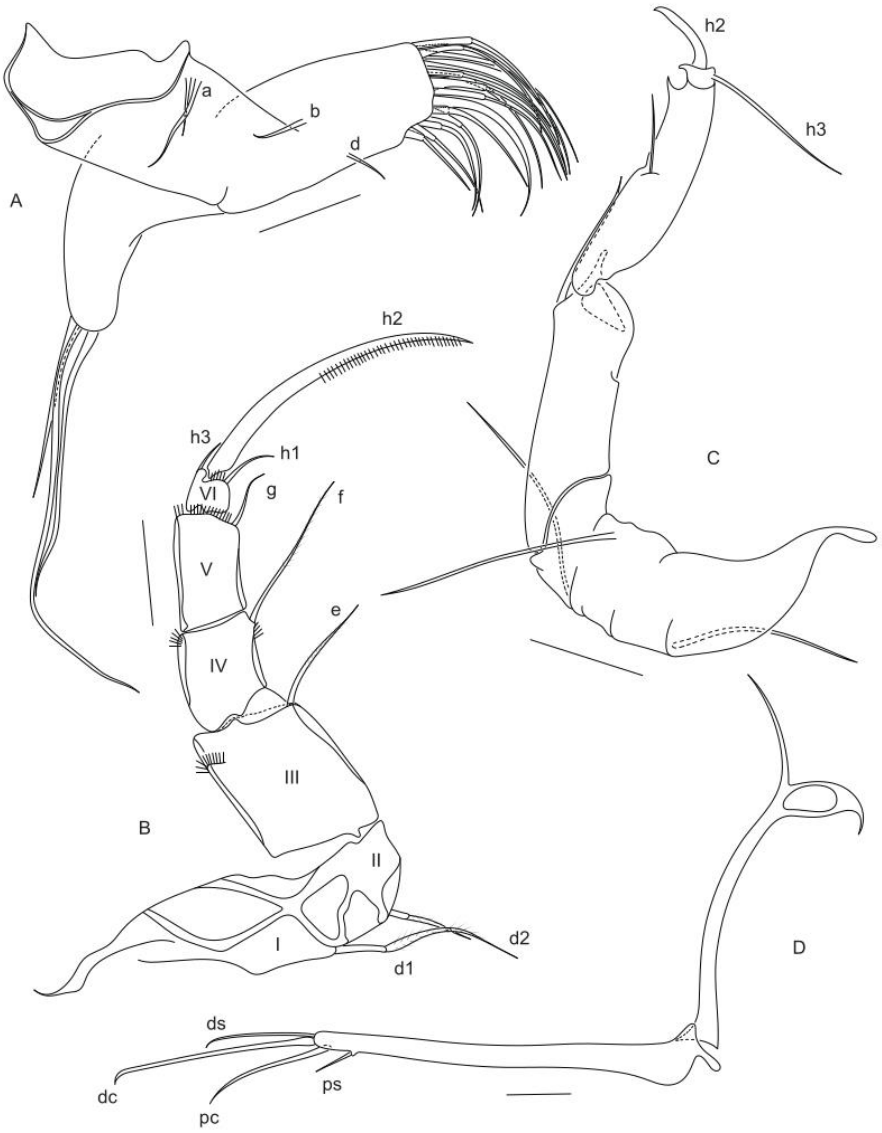


Fig. 5 - *Strandesia rondoniensis* **n**. **sp.**, female. A- first thoracic limb (JP160a); B- second thoracic limb (JP160a); C- third thoracic limb (JP125a); D- caudal ramus plus caudal ramus attachment (JP160a). Scale bars: A– 0.01mm, B, C, D– 0.05 mm.

3.2 Life cycle

Environmental parameters measured at the time and site of sampling were: pH = 6.4 ± 0.4; electrical conductivity = 84 µS cm^{-1} ± 6; preserved oxygen = 4.2 mg L^{-1} ± 1; and temperature = 29.5 C° ± 1.

Altogether, 11 newborn specimens of *S. rondoniensis* **n**. **sp.** were isolated and monitored for the completion of the life cycle, with their individual growth analyzed by

daily measuring the size of each individual over time. The duration of the experiment evaluated the longevity of the organisms, and the growth measurements were performed for 70 days, which was enough to observe the characteristics of the life cycle and establish the growth curve (Fig. 6).

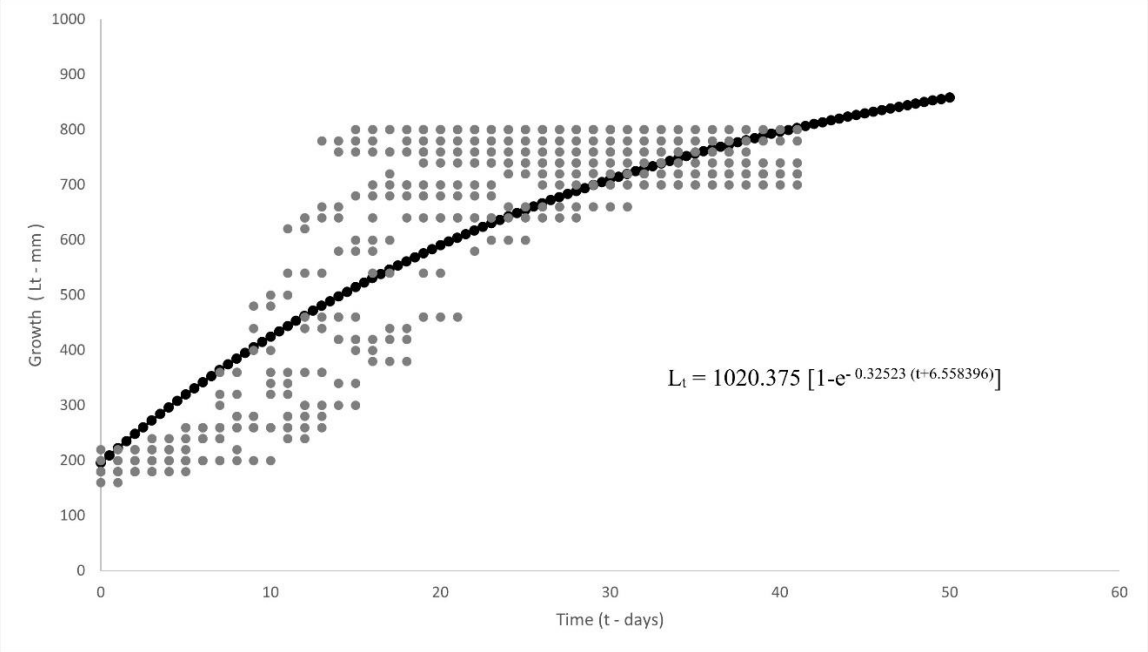


Fig. 6 - Individual growth curve of *Strandesia rondoniensis* **n**. **sp.** adjusted according to von Bertalanffy equation (1938), Temperature: 25 °C, light/dark photoperiod of 12:12 hours and feeding with 20 mL of Tetramin® fish food (2 g of solution in 1 liter of distilled water) and 20 mL of *Raphidocelis subcapitata* suspension (≈ 10^8 L^{-1} cells).

All observed individuals were female, indicating that parthenogenesis is the reproductive mode for this species. The life cycle experiment confirmed that *S. rondoniensis* **n**. **sp.** reproduces by parthenogenesis as all specimens remained isolated throughout the experiment and began to lay eggs after 21.27 ± 3.03 days.

The life cycle results show that *S. rondoniensis* **n**. **sp.** had an initial average size of 183.33 ± 14.35 µm and reached adulthood at approximately 23 to 28 days. The average size of adults was 771 ± 32.14 µm, the average longevity was 46.6 ± 11.71 days, and a maximum of 63 days. The first egg-laying occurred at 21.27 ± 3.03 days, and egg hatching between 2 and 4 days after laying. The hatchlings that hatched during the experiment were counted and removed. Thus, the average fecundity of the females was 176.45 ± 117.79 hatchlings per female, with a maximum of 327 and a minimum of 38 hatchlings

per female. The summary of data can be seen in Table 4. The individual growth curve for *S. rondoniensis* **n**. **sp.** is shown in Figure 6, and fertility data can be seen in Figure 7.

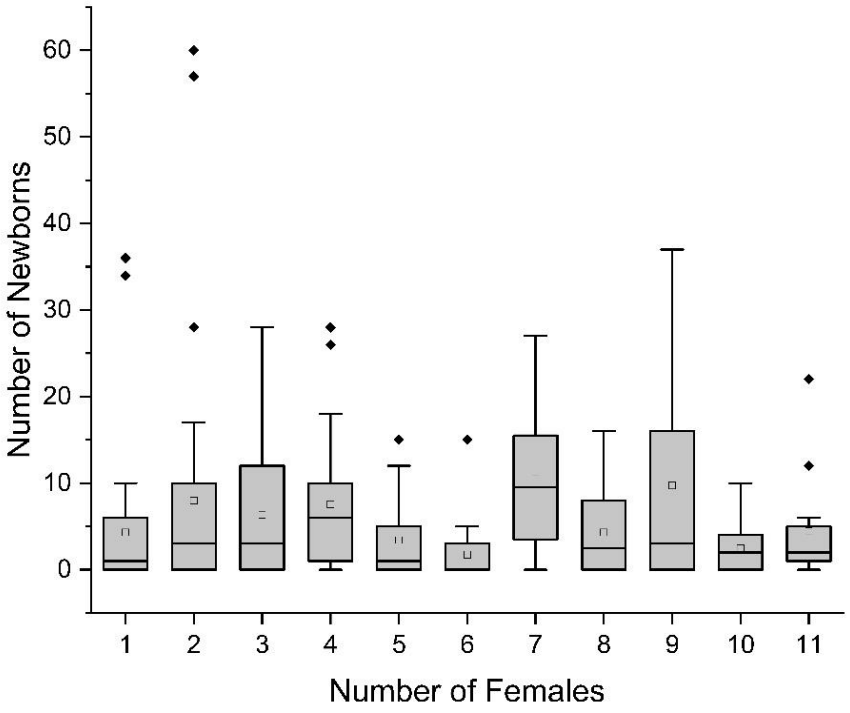


Fig. 7 - Average daily fecundity (n= 11) for *Strandesia rondoniensis* **n**. **sp.** during its life cycle when grown in the laboratory under controlled conditions.

Table 4 - Life cycle variables (mean ± standard deviation: n = 11) of ostracode *Strandesia rondoniensis* **n**. **sp.** cultured in the laboratory at 25±1°C, under a photoperiod of 12 h light: 12 h dark, and fed with 20 mL of Tetramin® fish food (2 g of solution in 1 liter of distilled water) and 20 mL of suspension of *Chlorella sorokiniana* (≈ 108 L^{-1} cells).

Life cycle parameters	Variables
Maximum length (μm)	800
Average adult length (μm)	771 ± 32,14
Mean newborn length (μm)	183,33 ± 14,35
Average length at first play (μm)	716 ± 69,17
Minimum length on first play (μm)	600
Average total fecundity (newborn per female over a lifetime)	176,45 ± 117,79
Maximum longevity (days)	63
Average longevity (days)	46,6 ± 11,71
Mean age of first reproduction (days)	21,27 ± 3.03

4. Discussion

4.1 Taxonomy

Ferreira, Higuti & Martens (2019) described *N. striata* for the subfamily Cypricercinae and stated that this species, along with *B. lineata* (Victor & Fernando, 1981) Savatenalinton & Martens 2009c, would constitute a case of evolutionary convergence. While similar at first sight in carapace morphology and ornamentation, their soft parts showed significant differences. The Brazilian species *S. rondoniensis* **n**. **sp.** described here configures another case of evolutionary convergence with those species studied by Ferreira, Higuti & Martens (2019).

Strandesia rondoniensis **n**. **sp.** shows high similarity with *N. striata* and *B. lineata* concerning carapace morphology. All three species have a characteristic subtriangular, tumid carapace with symmetry in the posterior contour, asymmetry in the anterior contour, and a relatively similar ornamentation pattern. The carapace internal view also shows similar morphology among these three species, with all of them exhibiting similar shapes on all margins and the presence of groove on the anterior calcified inner lamella: a crucial diagnostic trait differentiating genera within the subfamily Cypricercinae. Despite all these similarities, *S. rondoniensis* **n**. **sp.**, *B. lineata* and *N. striata* also show consistent, although subtle, differences on the carapace. These are summarized in Table 2. The soft parts, on the other hand, show noticeable differences.

Comparing the appendages of *S. rondoniensis* **n**. **sp.** with those of *N. striata*, the differences are high and allow an easy differentiation between these two species. On the other hand, the comparison with *B. lineata* requires attention as this pair is even more alike in carapace and appendage morphology. However, even so, the new species described here shows consistent differences in diagnostic characters used to differentiate species and genera within the subfamily Cypricercinae. All differences observed in appendages are summarized in Table 3 and fully detailed below.

Wouters organ on antennula

The presence of a Wouters organ is described and illustrated for *S. rondoniensis* **n**. **sp.**, described here. Concerning *B. lineata*, its original description (Victor & Fernando 1981), its redescription (Ferreira, Higuti & Martens 2019), or descriptions of species later considered as synonyms of *B. lineata* (i.e., *Paracypretta amati* Martens, 1984 and *Strandesia biwaensis* Okubo, 2004) do not mention this structure. However,

Savatenalinton & Martens (2010), who analyzed females of *B. lineata* from several localities, described the presence of the Wouters organ for this species (although illustrations were not provided). Therefore, we consider the presence of the Wouters organ known for *S. rondoniensis* **n**. **sp.** and *B. lineata*.

Concerning *N. striata*, there is no mention of the Wouters organ in the diagnosis of both the genus and the species, and the information is also not available either in the description or illustrations of the antennula. Then, the doubt about the presence of this structure in the species described by Ferreira, Higuti & Martens (2019) remains. As *S. rondoniensis* **n**. **sp.** and *B. lineata* both show the presence of the Wouters organ, we suggest reanalyzing *N. striata* material to clarify the similarities and differences between the three convergent species.

Rome organ on antennula

As occurs with the Wouters organ, among the three convergent species compared here, the presence of the Rome organ is known without doubt for *S. rondoniensis* **n**. **sp.** and *B. lineata*. Ferreira, Higuti & Martens (2019) did not describe or illustrate this structure for *N. striata* but stated that its antennula follows the typical pattern of the subfamily. In fact, for the 12 genera included in the Cypricercinae, at least six exhibit the trait, with variable lengths (Savatenalinton & Martens 2009c). The combination of the Rome and Wouters organs is also variable (Savatenalinton & Martens 2009c). Considering only the three genera composing the tribe Bradleystrandesiini, there are species with both organs - *Bradleytriebella tuberculata* (Hartmann, 1964) or with Rome organ but no Wouters organ - *Bradleystrandesia fuscata* (Jurine, 1820), *Spirocypris horrida* (Sars, 1926). Therefore, as the presence of these structures - combined or alone - can be one of the diagnostic characteristics effectively dividing genera within the subfamily, we stress the importance of clarifying their presence on *N. striata*.

Chaetotaxy of sixth segment on antennula

The chaetotaxy on the sixth segment of the antennula differs between *S. rondoniensis* **n**. **sp.** and *B. lineata*. Comparing the material described here with the illustrations provided for *P. amati* (Martens, 1984), the number of setae varies from 5 to 3. However, considering the original description of *B. lineata* (Victor & Fernando 1981), its abbreviated redescription (Savatenalinton & Martens 2010), or its full redescription

(Ferreira, Higuti & Martens 2019) do not mention or illustrate the chaetotaxy of the sixth segment, it is unclear whether this species indeed has two setae less or whether it was merely a misunderstanding due to the naturally confused state of antennular chaetotaxy.

The same occurs with *N. striata,* for which there is no detailed information about the antennula chaetotaxy in the description or illustrations. It is worth stressing here that, within the tribe Bradleystrandesiini, we found species with five setae on the sixth segment of antennula (*B. tuberculata*, see Savatenalinton & Martens, 2009c, fig. 28A), with four setae (*B. fuscata*, see Savatenalinton & Martens, 2009c, fig. 18A) or with three setae (*Bradleytriebella decorata* (Sars, 1903), see Savatenalinton & Martens, 2010, fig. 44A). Considering this variation naturally found on representatives of the tribe, we find it essential to clarify this antennular chaetotaxy for both *B. lineata* and *N. striata*.

Aesthetasc Y on antenna

The aesthethasc Y assumes a particular characteristic in *B. lineata*, reaching the first endopodal segment apex (see Martens 1984, fig.99) or even exceeding it (see Victor & Fernando 1981, fig.97, Ferreira, Higuti & Martens 2019, fig.6A). This unusually long length is consistent throughout the literature dealing with *B. lineata*, from the original description (Victor & Fernando, 1981) through the redescriptions (Savatenalinton & Martens 2010, Ferreira, Higuti & Martens, 2019) to species later synonymized with it (Martens, 1984). Besides that, within the Cypricercinae, this greater length is known only for *B. lineata* (Ferreira, Higuti & Martens, 2019). We consider it then safe to affirm that this is a particularity of the species *B. lineata* itself and can, therefore, be used as a diagnostic character for it. *S. rondoniensis* **n**. **sp.**, on the other hand, has an aesthethasc Y considered normal by applying the size-parameters indicated by Ferreira, Higuti & Martens (2019) and, therefore, resembles the one found in *N. striata* and departs from those found in *B. lineata*.

Chaetotaxy of first palp segment on maxillula

This taxonomic character is extremely conservative throughout the Cypricercinae. Only two of the 12 genera within this subfamily differ from the usual pattern of 6 apical setae plus a subapical inserted seta (Savatenalinton & Martens 2009c, Ferreira, Higuti & Martens 2019). *N. striata* is one of these two, with a distinct pattern of 4 apical setae plus

a subapical seta (Ferreira, Higuti & Martens 2019). It is worth noting that Martens (1984) illustrated a pattern of 5+1 setae on the first maxillular palp. However, since this is not consistent with other descriptions of *B. lineata* material (Ferreira, Higuti & Martens, 2019) and considering this chaetotaxy is hard to visualize, we consider here that *B. lineata* follows the dominant and conservative Cypricercinae pattern of 6+1 setae, as well as *S. rondoniensis* **n. sp.**

Chaetotaxy of second palp segment on maxillula

The chaetotaxy of the second segment of the palp on maxillula is also conservative, and generally consists of 3 claws plus 3 setae (Savatenalinton & Martens 2009c, Ferreira, Higuti & Martens 2019). In fact, 9 from 12 genera within Cypricercinae follow this pattern.

The exceptions include just three genera: *Neostrandesia* is one of them with a claw and 3 setae (Ferreira, Higuti & Martens, 2019). Martens (1984) illustrated a pattern of 1 claw plus 3 setae for *P. amati*, which would place *B. lineata* as another exception within the subfamily and approximate *B. lineata* from *N. striata*. However, other descriptions of *B. lineata* illustrated the general pattern (3+3) (Savatenalinton & Martens 2009c; Ferreira, Higuti & Martens, 2019). Once again, considering the difficulty in visualizing the maxillular chaetotaxy, we consider that both *S. rondoniensis* **n. sp.** and *B. lineata* follow the conservative pattern of 3 claws + 3 setae.

Sideways-directed bristles on maxillula

Within the Cypricercinae, the sideways-directed bristles are either absent or present as one or two unequal setae (Ferreira, Higuti & Martens, 2019). *N. striata* and *B. lineata* differ, whereby the former shows two and the latter just one sideways-directed bristles (Ferreira, Higuti & Martens, 2019). *S. rondoniensis* **n. sp.**, with two unequal sideway-directed bristles, is closer to *N. striata* than to *B. lineata* in this respect. It is worth noting that Savatenalinton & Martens (2010) indicated the presence of two sideways-directed bristles in their *B. lineata* abbreviated redescription. However, considering that the authors did not provide an illustration of this trait and Ferreira, Higuti & Martens (2019) later described and illustrated just one sideway-directed bristle for this species, we opt here to consider *B. lineata* maxillula with one sideway-directed bristle, instead of two. However, future clarification of this inconsistency is encouraged as this trait is relevant to diagnosing the different genera within the Cypricercinae subfamily.

'b' seta on first thoracic limb

Following the size parameters proposed by Ferreira, Higuti & Martens (2019), the b-seta on the first thoracic limb (when present) can be classified as long or giant, according to its length relative to the length of the apical setae on this appendage. *N. striata* stands out from all other representatives of the subfamily Cypricercinae as it is the only species known to have a giant b-seta (Ferreira, Higuti & Martens, 2019). *S. rondoniensis* **n. sp.** and *B. lineata* have a long b-seta (Victor & Fernando, 1981; Martens, 1984; Ferreira, Higuti & Martens, 2019) as it seems to be the pattern within Cypricercinae (Ferreira, Higuti & Martens, 2019).

'd' seta on first thoracic limb

The d-seta is usually used as a diagnostic character within Cypricercinae (Savatenalinton & Martens, 2009c). However, it has been demonstrated that the presence or absence of this trait does not follow a pattern that allows the classification of genera and tribes based on it (Ferreira, Higuti & Martens, 2019). The d-seta is present in *N. striata* (Ferreira, Higuti & Martens, 2019) and *S. rondoniensis* **n. sp.** On the other hand, for *B. lineata*, the d-seta is absent. In the original description of the species, Victor & Fernando (1981) described the presence of the d-seta. However, analyzing the figure provided by the authors, they misinterpreted one of the apical setae as being a d-seta (see Victor & Fernando 1981, fig.104), as discussed by Savatenalinton & Martens (2010). It is also worth noting that all later manuscripts dealing with *B. lineata* material (Martens 1984; Savatenalinton & Martens 2010; Ferreira, Higuti & Martens, 2019) indicate the absence of a d-seta for this species. Thus, we consider it an important diagnostic trait splitting *B. lineata* from both *N. striata* and *S. rondoniensis* **n. sp.**

4.2 Life cycle

The mean body size values obtained in this study for individuals of *S. rondoniensis* **n. sp.** was 771 ± 32.14 mm, close to the values presented by Pinto (1965) for the congeneric species *Strandesia trispinosa*. Freshwater ostracods can perform sexual reproduction and parthenogenesis, as well as produce resistant eggs in unfavorable environmental conditions (Butlin, Schön & Martens, 1998; Gandolfi et al., 2001; Pinto, Rocha & Martens, 2007; Martens et al., 2008).

Females reproduced parthenogenetically throughout the experiment, and no males appeared in the laboratory cultures. Moreover, the first reproduction occurred after 21.27 ± 3.03 days, and the post-laying development time varied between 2 and 4 days. Contrastingly, studies conducted by Havel, Barriel & Talbott (1995) verified a high variation of 1 to 157 days in egg development time for *Heterocypris incongruens*. Ikeya & Kato (2000) reported that egg development time for *Xestoleberis hanaii* lasted 9 days and took about 33 days to reach adulthood. Mezquita, Olmos & Oltra (2000) verified that *Cyprideis torosa* individuals reached adult size after an average of 154 days.

Currently, the number of experimental studies about ostracod life cycles is still low, especially compared to the amount of data available for other taxonomic groups, such as cladocerans (Mount & Norberg, 1984; Santos-Wisniewski, Rocha & Matsumura-Tundisi, 2006; Silva et al. 2014; Silva et al. 2023), rotifers (Snell, Moffat, 1992; Moreira et al. 2016), copepods (Ferrari & Ascolini, 1975; Peterson, 1998; Melão & Rocha, 2004), among others. The life cycle data for ostracods available in the literature typically use the total number of eggs as the base to calculate the fecundity rates. In this manner, McGregor (1972) indicated that *Cypria turneri* presented low fertility rates, producing no more than 8 eggs per female. Gandolfi et al. (2001) reported that *H. incongruens* presented an average of 2.5 to 6 eggs per day; Notaro (1998) reported an average of 1.2 to 1.5 eggs per day for *Eucypris virens*. Yin (1997) indicated a range of 0.5 to 7 eggs per day for the species *Limnocythere inopinata*, and according to McLay (1978), both *Herpetocypris reptans* and *Cyprinotus carolinensis* laid about 0.5 eggs per day. Pinto, Rocha and Martens (2007) reported that *Penthesilenula brasiliensis* presented an average egg production between 5 and 6. Furthermore, Gandolfi et al. (2001) conducted a laboratory experiment using the species *Darwinula stevensoni* and found that each female produced up to 12 juveniles per litter. Meanwhile, Ikeya & Kato (2000) studied the life cycle of *Xestoleberis hanaii*, noting that each female produced between 39 and 94 juveniles in all. Kawamata et al. (2018) showed that *Euphilomedes nipponica* generated up to 120 descendants. The daily average of new juveniles for *S. rondoniensis* **n**. **sp.** was 5.7 ± 2.91, while the total average was 176.45 ± 117.79 offspring per female, which are higher values than most fecundity data presented above. Observations reported by Gandolfi et al. (2001) verified that new eggs were produced continuously, resulting in clutches with embryos in different stages of development, and the same pattern was observed in this study.

It is important to emphasize that although the number of eggs was considered and counted in this study, there was a discrepancy between the number of eggs and the number

of new juveniles counted during the experiment. According to Havel, Barriel & Talbott (1995), although it is possible to lay eggs individually, females tend to deposit their eggs mainly in groups, overlapping the eggs between the egg masses themselves and making it hard to visualize each egg and count correctly. Therefore, we used the final number of juveniles hatched during the experiment to calculate the fertility rates for all females used in this study.

Heterocypris incongruens is the only ostracod species currently standardized for ecotoxicological studies (ISO 14371, 2012) focused on reproduction (Barriel & Talbott 1995; Hiki et al. 2017). *S. rondoniensis* **n**. **sp.** exhibited higher fertility rates than the standardized species. Longevity follows the same pattern, with *S. rondoniensis* **n**. **sp.** showing an average and maximum of 46.6 and 63 days, respectively, which is higher than that presented by Hiki et al. (2017) of 37 days for *H. incongruens.*

In recent decades, interest in using ostracods as test organisms has increased (Khangarot & Das, 2009; Shuhaimi-Othman et al., 2011; Lima et al., 2019) for several reasons. First, ostracods are small and can be easily used in small-scale assessments (Chen et al. 2022). In addition to that, they have a wide geographic distribution and can be found in abundance in both marine and freshwater environments in benthic habitats or associated with aquatic vegetation (Tressler, 1959; Havel, Barriel & Talbott, 1995). Moreover, in many cases, they reproduce by parthenogenesis (Havel & Hebert, 1993), allowing the establishment of isofemale populations (clones), which has the advantage of keeping low genetic variability in the population throughout the experiments (Havel & Hebert, 1989), and performing life history studies exclusively with female populations (Havel, Barriel & Talbott, 1995). In addition to these characteristics, many ostracod species are easily maintained for a long time in the laboratory (Havel & Hebert, 1989; Hiki et al. 2017; Lima et al. 2019). Our results about the life cycle of *S. rondoniensis* **n**. **sp.** show its potential as a test organism in laboratory studies as it has fast growth and egg maturation, high fertility rates, and is easily cultivated and maintained.

5. Conclusion

The present study describes a new ostracod species of the genus *Strandesia* consisting of a third element of the evolutionary convergence documented by Ferreira, Higuti & Martens (2019) within Cypricercinae. Morphology indicates that the new species belongs within *Strandesia* despite the resemblance to *B. lineata* and *N. striata.* Further morphological and molecular divergence assessment among the three convergent species is recommended. Finally, the life cycle results suggested that *S. rondoniensis* **n. sp.** has excellent potential for future ecotoxicological studies due to easy laboratory adaptability and rapid growth and reproduction.

References

Adamowicz, S. J., (2015). International Barcode of Life: Evolution of a global research community. *Genome*, *58*(5), 151-162. https://doi.org/10.1139/gen-2015-0094

Adolfsson, S., Michalakis, Y., Paczesniak, D., Bode, S.N.S. Butlin, R.K., Lamatsch, D.K., Martins, M.J.F. Schmit, O., Vandekerkhove, J., Jokela, J., (2010). Evaluation of elevated ploidy and asexual reproduction as alternative explanations for geographic parthenogenesis in *Eucypris virens* ostracods. *Evolution*, 64: 986-997. https://doi.org/10.1111/j.1558-5646.2009.00872.x

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS (ABNT). (2016). **NBR 12713:** Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com Daphnia spp (Crustacea, Cladocera). Rio de Janeiro.

Butlin, R., Schön, I., & Martens, K., (1998). Asexual reproduction in nonmarine ostracods. *Heredity*, *81*(5), 473-480. https://doi.org/10.1046/j.1365-2540.1998.00454.x

Chen, L., Huo, Z., Su, C., Liu, Y., Huang, W., Liu, S., & Sui, Q., (2022). Sensitivity of Ostracods to U, Cd and Cu: The Case of *Cypridopsis vidua*. *Toxics*, *10*(7), 349. https://doi.org/10.3390/toxics10070349

Coviaga, C., Cusminsky, G., Baccalá, N., Pérez, A. P., (2015). Dynamics of ostracod populations from shallow lakes of Patagonia: life history insights. *Journal of Natural History*, *49*(17-18), 1023-1045. https://doi.org/10.1080/00222933.2014.981310

Cohen, A. C., (1983). Rearing and postembryonic development of the myodocopid ostracode *Skogsbergia lerneri* from coral reefs of Belize and the Bahamas. *Journal of Crustacean Biology*, *3*(2), 235-256. https://doi.org/10.2307/1548260

Dole-Olivier, M. J., Galassi, D. M. P., Marmonier, P., & Creuzé des Châtelliers, M., (2000). The biology and ecology of lotic microcrustaceans. *Freshwater biology*, *44*(1), 63-91. https://doi.org/10.1046/j.1365-2427.2000.00590.x

dos Santos Lima, J. C., Moreira, R. A., Neto, A. J. G., Freitas, E. C., Daam, M. A., Rocha, O., (2023). Toxicity of Binary Metal Mixtures to the Tropical Ostracod *Strandesia trispinosa*. *Bulletin of Environmental Contamination and Toxicology*, *110*(3), 64. https://doi.org/10.1007/s00128-023-03704-w

dos Santos Lima, J. C., Neto, A. J. G., de Pádua Andrade, D., Freitas, E. C., Moreira, R. A., Miguel, M., Rocha, O., (2019). 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*, *180*, 535-541. https://doi.org/10.1016/j.ecoenv.2019.05.018

Felsenstein J., (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791 https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Ferrari, I., & Ascolini, A., (1975). Life cycle of *Eudiaptomus intermedius* (Steuer) (Copepoda, Calanoida) in a mountain lake. *Italian Journal of Zoology*, *42*(1), 39-47. https://doi.org/10.1080/11250007509430129

Ferreira, V. G., Higuti, J., Martens, K., (2019). A striking case of convergent evolution in two species of Cypricercinae (Crustacea, Ostracoda), with the description of a new genus and species from Brazil. *Zoologischer Anzeiger*, 283: 1–11. https://doi.org/10.1016/j.jcz.2019.06.007

Freitas, E. C., & Rocha, O., (2006). The life cycle of *Pseudosida ramosa*, Daday 1904, an endemic Neotropical cladoceran. *Acta Limnologica Brasiliensia*, *18*(34), 293-303.

Gandolfi, A., Todeschi, E., Rossi, V., & Menozzi, P., (2001). Life history traits in *Darwinula stevensoni* (Crustacea: Ostracoda) from Southern European populations under

controlled conditions and their relationship with genetic features. *Journal of Limnology*, *60*(1), 1-10.

Havel, J. E., & Hebert, P. D., (1989). Apomictic parthenogenesis and genotypic diversity in *Cypridopsis vidua* (Ostracoda: Cyprididae). *Heredity*, *62*(3), 383-392. https://doi.org/10.1038/hdy.1989.53

Havel, J. E., & Hebert, P. D. N., (1993). Clonal diversity in parthenogenetic ostracods. *Ostracoda in the earth and life sciences*, 353-368.

Havel, J. E., & Talbott, B. L., (1995). Life history characteristics of the freshwater ostracod *Cyprinotus incongruens* and their application to toxicity testing. *Ecotoxicology*, *4*, 206-218. https://doi.org/10.1007/BF00116482

Higuti, J., Roche, K. F., & Martens, K., (2017). Checklist de ostrácodes (Crustacea, Ostracoda) dulcícolas do Pantanal Sul Mato-grossense, Brasil. *Iheringia. Série Zoologia*, *107*, e2017114. https://doi.org/10.1590/1678-4766e2017114

Higuti, J., Schön, I., Audenaert, L., Martens, K., (2013). On the *Strandesia obtusata*/*elliptica* lineage (Ostracoda, Cyprididae) in the alluvial valley of the upper Paraná River (Brazil), with the description of three new species. *Crustaceana*, *86*(2), 182-211. https://doi.org/10.1163/15685403-00003160

Hiki, K., Tobino, T., Nakajima, F., Tsukahara, K., (2017). Duration of life-cycle toxicity tests with the ostracod *Heterocypris incongruens*. *Environmental toxicology and chemistry*, *36*(12), 3443-3449. https://doi.org/10.1002/etc.3931

Horne, D. J., Cohen, A. & Martens, K., (2002). Taxonomy, morphology and biology of quaternary and living Ostracoda. In: Holmes, J.A. & Chivas, A.R. (Eds.), The Ostracoda applications in Quaternary research. American Geophysical Union, Washington, DC, pp. 5–36. https://doi.org/10.1029/131GM02

Kesling, R. V., (1949). *The morphology of ostracod molt stages*. University of Illinois at Urbana-Champaign. Monogr.21, 1-126.

Ikeya, N., & Kato, M., (2000). The life history and culturing of *Xestoleberis hanaii* (Crustacea, Ostracoda). *Hydrobiologia*, *419*, 149-159. https://doi.org/10.1023/A:1003931901501

International Organization for Standardization - ISO, (2012). Water Quality — Determination of Freshwater Sediment Toxicity to *Heterocypris incongruens* (Crustacea, Ostracoda), first ed. The International Organization for Standardization, Geneva, Switzerland, pp. 14371 (ISO 14371:2012(E)).

Kawamata, K., Sato, M., & Abe, K., (2018). Complete life cycle of the ostracod *Euphilomedes nipponica* (Myodocopida, Philomedidae). *Plankton and Benthos Research*, *13*(2), 83-89. https://doi.org/10.3800/pbr.13.83

Khangarot, B. S., & Das, S., (2009). Acute toxicity of metals and reference toxicants to a freshwater ostracod, *Cypris subglobosa* Sowerby, 1840 and correlation to EC50 values of other test models. *Journal of hazardous materials*, *172*(2-3), 641-649. https://doi.org/10.1016/j.jhazmat.2009.07.038

Koenders, A., Martens, K., Halse, S., & Schoen, I., (2012). Cryptic species of the *Eucypris virens* species complex (Ostracoda, Crustacea) from Europe have invaded Western Australia. *Biological Invasions*, *14*, 2187-2201. https://doi.org/10.1007/s10530-012-0224-y

Koenders, A., Schön, I., Halse, S., Martens, K., (2017). Valve shape is not linked to genetic species in the *Eucypris virens* (Ostracoda, Crustacea) species complex. *Zoological Journal of the Linnean Society*, *180*(1), 36-46. https://doi.org/10.1111/zoj.12488

Martens, K., (1984). On the freshwater ostracods (Crustacea: Ostracoda) of the Sudan, with special reference to the Red Sea Hills, including a description of a new species. *Hydrobiologia*, 110: 137–161. https://doi.org/10.1007/BF00025786

Martens, K., Schön, I., Meisch, C., Horne, D. J., (2008). Global diversity of ostracods (Ostracoda, Crustacea) in freshwater. *Hydrobiologia*, *595*, 185-193. https://doi.org/10.1007/978-1-4020-8259-7_20

Mansano, A. S., Moreira, R. A., Dornfeld, H. C., Diniz, L. G., Vieira, E. M., Daam, M. A., Rocha, O. Seleghim, M. H., (2018). Acute and chronic toxicity of diuron and carbofuran to the neotropical cladoceran *Ceriodaphnia silvestrii*. *Environmental Science and Pollution Research*, *25*, 13335-13346. https://doi.org/10.1007/s11356-016-8274-9

McGregor, D. L., (1972). The Ostracoda of Gull Lake, Michigan: selected aspects of their ecology. Ph.D. Thesis, Michigan State Univ., East Lansing, 187 pp.

Meisch, C., Smith, R. J., Martens, K., (2019). A subjective global checklist of the extant non-marine Ostracoda (Crustacea). *European Journal of Taxonomy*, 492: 1–135. https://doi.org/10.5852/ejt.2019.492

Melão, M. D. G. G., & Rocha, O., (2004). Life history, biomass and production of two planktonic cyclopoid copepods in a shallow subtropical reservoir. *Journal of Plankton Research*, *26*(8), 909-923. https://doi.org/10.1093/plankt/fbh080

Mesquita-Joanes, F., Smith, A. J., Viehberg, F. A., (2012). The ecology of Ostracoda across levels of biological organization from individual to ecosystem: a review of recent developments and future potential. *Developments in Quaternary Sciences*, *17*, 15-35. https://doi.org/10.1016/B978-0-444-53636-5.00002-0

Mariani, L., Grenni, P., Caracciolo, A. B., Pescatore, T., Spataro, F., Rauseo, J., Patrolecco, L., (2022). Use of the *Heterocypris incongruens* bioassay for assessing ecotoxicity of soils containing the anionic surfactant sodium lauryl ether sulfate (SLES). *Ecological Indicators*, *145*, 109597. https://doi.org/10.1016/j.ecolind.2022.109597

McLay, C. L., (1978). Comparative observations on the ecology of four species of ostracods living in a temporary freshwater puddle. *Canadian Journal of Zoology*, *56*(4), 663-675. https://doi.org/10.1139/z78-094

Mount, D. I., & Norberg, U. T., (1984). A seven-day life cycle cladoceran toxicity test. *Environmental Toxicology and Chemistry: An International Journal*, *3*(3), 425-434. https://doi.org/10.1002/etc.5620030307

Moreira, R. A., Mansano, A. S., Rocha, O., (2016). Life cycle traits of *Philodina roseola* Ehrenberg, 1830 (Rotifera, Bdelloidea), a model organism for bioassays. *Anais da*

Academia Brasileira de Ciências, *88*, 579-588. https://doi.org/10.1590/0001-3765201620140729

Niyommaneerat, W., Nakajima, F., Tobino, T., Yamamoto, K., (2017). Development of a chronic sediment toxicity test using the benthic ostracod *Heterocypris incongruens* and their application to toxicity assessments of urban road dust. *Ecotoxicology and environmental safety*, *143*, 266-274. https://doi.org/10.1016/j.ecoenv.2017.05.011

Notaro, A., (1998). *Variazioni del ciclo biologico in cloni di Eucypris virens (Crustacea: Ostracoda)*. 1st Degree thesis, Università di Parma.

Okubo, I., (2004). Nihon tansui san kaimijinko rui ni tsuite. *Kabushikigaisha Sanmon Insatsusho, Okayama, Japan*, *72*.

Peterson, W., (1998). Life cycle strategies of copepods in coastal upwelling zones. *Journal of Marine Systems*, *15*(1-4), 313-326. https://doi.org/10.1016/S0924-7963(97)00082-1

Pinto, I. D., (1965). *A new fresh-water ostracode Cyprinotus trispinosus Pinto et Purper, sp. nov., from southern Brazil, its ontogenetic carapace variation and seasonal distribution* (No. 7). Escola de Geologia, Universidade do Rio Grande do Sul.

Pinto, R. L., Rocha, C. E., & Martens, K., (2007). Early release of eggs and embryos in a brooding ancient asexual ostracod: brood selection or a gambling strategy to increase fecundity? *Hydrobiologia*, *585*, 249-253. https://doi.org/10.1007/s10750-007-0641-6

Santos-Wisniewski, M. J., Rocha, O., Matsumura-Tundisi, T. (2006). Aspects of the life cycle of *Chydorus pubescens* Sars., 1901 (Cladocera, Chydoridae). *Acta Limnologica Brasiliensia*, v. 18, n. 3, p. 305-310,

Savatenalinton, S., Martens, K., (2009b). Redescription of the type species of *Strandesia* Stuhlmann, 1888 and *Cypricercus* Sars, 1895 (Crustacea, Ostracoda, Cypricercinae), with a description of a new species of *Cypricercus* from South Africa. *Zootaxa*, 2007: 1–42. https://doi.org/10.11646/zootaxa.2007.1.1

Savatenalinton, S., Martens, K., (2009c). Generic revision of Cypricercinae McKenzie, 1971 (Crustacea, Ostracoda), with the description of three new genera and one new

species and a phylogenetic analysis of the subfamily. *Hydrobiologia*, 632: 1–48. https://doi.org/10.1007/s10750-009-9826-5

Savatenalinton, S., Martens, K., (2010). On the subfamily Cypricercinae McKenzie, 1971 (Crustacea, Ostracoda) from Thailand, with the description of six new species. *Zootaxa*, 2379: 1–77. Doi: 10.11646/ZOOTAXA.2379.1.1

Sevilla, J. B., Nakajima, F., & Kasuga, I., (2014). Comparison of aquatic and dietary exposure of heavy metals Cd, Cu, and Zn to benthic ostracod *Heterocypris incongruens*. *Environmental Toxicology and Chemistry*, *33*(7), 1624-1630. https://doi.org/10.1002/etc.2596

Schmidt, R. E., Shoobs, N. F., & McMullin, E. R., (2018). Occurrence of the large ostracod, *Chlamydotheca unispinosa* (Baird, 1862), in temporary waters of Montserrat, Lesser Antilles. *ZooKeys*, (748), 89. 10.3897/zookeys.748.22323

Schön, I., Higuti, J., Patel, T., & Martens, K., (2018). Aquatic long-distance dispersal and vicariance shape the evolution of an ostracod species complex (Crustacea) in four major Brazilian floodplains. *Molecular Phylogenetics and Evolution*, 121, 86-97. https://doi.org/10.1016/j.ympev.2017.12.019

Schön, I., Pinto, R.L., Halse, S., Smith, A.J., Martens, K., Birky, C.W., (2012). Cryptic species in putative ancient asexual darwinulids (Crustacea, Ostracoda) *Plos ONE*, 7, e39844. https://doi.org/10.1371/journal.pone.0039844

Shuhaimi-Othman, M., Yakub, N., Ramle, N. A., Abas, A., (2011). Toxicity of metals to a freshwater ostracod: *Stenocypris major*. *Journal of Toxicology*, *2011*. https://doi.org/10.1155/2011/136104

Snell, T. W., & Moffat, B. D., (1992). A 2-d life cycle test with the rotifer *Brachionus calyciflorus*. *Environmental Toxicology and Chemistry: An International Journal*, *11*(9), 1249-1257. https://doi.org/10.1002/etc.5620110905

Steinberg, C. E., & Steinberg, C. E., (2018). Dietary restriction, starvation, compensatory growth 'short-term fasting does not kill you: It can make you stronger'. *Aquatic Animal Nutrition: A Mechanistic Perspective from Individuals to Generations*, 137-287. https://doi.org/10.1007/978-3-319-91767-2_4

Strayer, D., (1988). Life history of a lacustrine ostracod. *Hydrobiologia*, *160*, 189-191. https://doi.org/10.1007/BF00015482

Sparre, P., (1998). Introduction to tropical fish stock assessment. Part 1. Manual. *FAO Fish. Tech. Paper.*, *306*, 1-407.

Tressler, W. L., (1959). Ostracoda. In W. T. Edmondson (ed.), Fresh-Water Biology. J. Wiley & Sons, N.Y.: 657-734.

Victor, R., Fernando, C. H., (1981). Freshwater Ostracods (Crustacea: Ostracoda) of the genus *Strandesia* Vavra, 1895 from Malaysia, Indonesia and the Philippines. *Archiv für Hydrobiologie*, 58: 469–522.

Vinyard, G., (1979). An ostracod (*Cypridopsis vidua*) can reduce predation from fish by resisting digestion. *American Midland Naturalist*, 188-190. https://doi.org/10.2307/2425084

YIN, Y., (1997). *Contribution to the morphology and ecology of ostracoda Limnocytheridae and Candonidae: Comparative studies between Austrian and Chinese Crustacea.* PhD Thesis, University of Vienna, 163p

Chapter 3 - Toxicity of isolated and mixed metals to a native Amazonian ostracod and ecological risk assessment

Submitted in Environmental Science and Pollution Research (Manuscript Number: ESPR-D-24-01603)

Toxicity of isolated and mixed metals to a native Amazonian ostracod and ecological risk assessment

Abstract

In recent decades the Amazonian ecosystem has received large amounts of domestic and industrial effluents, as well as mining-related waste contributing significant quantities of metal to water bodies. Thus, the main objective of the study was to verify the sensitivity of a native Amazonian ostracod (*Standesia rondoniensis*) species to isolated and mixed metal salts ($CuSO_4$; $ZnCl_2$; $CdCl_2$ and $HgCl_2$). The sensitivity will be compared to other species using species sensitivity distributions (SSDs) for an ecological risk assessment (ERA). The experiment consisted of simultaneously exposing each metal salt alone and in mixture, through a factorial design for toxicity with 25 different combinations for 48 hours. For the ERA, metal concentrations measured in the water of various aquatic environments in the Amazon basin were considered based on the risk quotient values. The results showed that the metal toxicity gradient was Cd>Hg>Cu>Zn, respectively. The toxicity in the mixture showed that the combination of Cu-Cd and Cu-Zn better fit the model (CA), indicating mainly synergism when copper predominated in the mixture. Meanwhile, the Cu-Hg interaction fit the model better (IA), either, indicating synergism when copper was at a higher concentration. The ERA showed a high risk (RQ>1) for the Cd, Cu, Hg metals.

Keywords: Acute toxicity, aquatic invertebrate, Metal contamination, Risk assessment *Strandesia rondoniensis*, Water pollution

1. Introduction

The increased occurrence and concentration of potentially toxic metals from anthropogenic sources pose a serious threat to the health of Amazonian aquatic ecosystems (Gomes et al. 2023; Moulatlet et al. 2023). In recent decades, the increase in human migratory flow to the Amazon region has led to the expansion of agriculture, livestock, industry (Rico et al. 2022; Cabrera et al. 2023; Rizzi et al. 2023), and mining operations (Capparelli et al. 2020; Moulatlet et al. 2023), in addition to the growth of urban areas with little sanitation infrastructure (Gomes et al. 2023). These activities have caused substantial damage to the aquatic ecosystems due to the continuous release of metals and other pollutants (Viana et al. 2021; Silva et al. 2023). Among the metals often reported at high concentrations, cadmium (Cd) (Naka et al. 2020), copper (Cu) (Araújo et al. 2022, zinc (Zn), and mercury (Hg) (Crespo-Lopez et al. (2023) are outstanding pollutants.

Some recent studies have shown high concentrations of metals in freshwater environments from different regions of the Amazon region (Araujo et al. 2020; Moulatlet et al. 2023). In a recent review, Gomes et al. (2023) reported that 44% of studied sites presented metal concentrations above the limits established by Brazilian Environmental Legislation. These data demonstrate a problematic scenario as metals may present high toxicity and persistence in the environment (Wu et al. 2016; Yuan et al. 2017; Vatandoost et al. 2018; Ali et al. 2019). Several studies have corroborated that the entry of pollutants into the environment threats the preservation of aquatic biota (Rocha et al. 2018; Vatandoost et al. 2018; Ali et al. 2019). Since metals have different ways of interaction with organisms, they can cause deleterious effects such as changes in physiological processes (Jaishankar et al. 2014; Morroni et al. 2023) and reproductive performance (Taslima et al. 2022), DNA (Wu et al. 2016; Watson et al. 2018) and behavioral changes (Zhang et al. 2023), leading to populational declines (Tchounwou et al. 2012).

Currently, it is very well established that the simultaneous entry of several metals into aquatic ecosystems occurs (Moulatlet et al. 2023; Elnabi et al. 2023), and the interaction between pollutants can alter their toxicity in different ways, but generally, through synergistic effects on organisms (Altenburger et al. 2013; Li, Liu, Slaveykova, 2020). Arreguin-Rebello et al. (2024) also observed synergistic effects when exposing the rotifer *Proales similis* to different combinations of metals (Cu, Cd, Hg). Meng et al. (2008) verified synergistic effects for all combinations of metals they tested (Hg, Cd, Cu, Pd and Cr) in acute and chronic exposures with *Daphnia magna*. However,

ecotoxicological studies still need to be conducted to better understand the individual and combined effects of these pollutants on other aquatic organisms, mainly from tropical areas (McKinley et al. 2019; Jeong et al. 2023).

Although ecotoxicological studies have already used different tropical species (Bertoletti, 2009; Freitas and Rocha, 2014; Lima et al. 2019), few Amazonian species have been included as test organisms compared to individuals from other tropical areas or temperate regions (Daam and Van Den Brink, 2010). Ostracods are small benthic crustaceans, which represent one of the most diverse taxonomic groups in aquatic ecosystems (Horne et al. 2002; Coviaga et al. 2015). In recent ecotoxicological studies, ostracods have been used as test organisms (Khangarot & Das, 2009; Mariani et al. 2022; Chen et al. 2022; Lima et al. 2019, 2023) due to their biological characteristics, easy cultivation on a small scale, rapid acclimatization, and adaptation to laboratory conditions, and sensitivity (Havel & Hebert, 1989; Khangarot & Das, 2009; Wang, 2022). Despite the ecological significance of ostracods and their sensitivity to various compounds, there is still a gap in toxicity studies in the Neotropical region.

In line with the aspects described above, the present study aimed to: I) Determine the sensitivity of *Strandesia rondoniensis* (Cyprididae) a ostracod Neotropical species recently described by Gomes et al. (2024) (submitted article), to the metals salts copper sulfate ($CuSO_4$), cadmium chloride ($CdCl_2$), zinc chloride ($ZnCl_2$) and mercuric chloride ($HgCl_2$), through acute toxicity tests (48 h), both, isolated and in mixtures, evaluating the survival of the organisms; II) Compare sensitivity with other species (from different taxonomic groups) through species sensitivity distribution curves , and III) assess the ecological risk associated to those metals in the Amazon context. Thus, in this work we contribute for elucidating the consequences for ostracods and other taxonomic groups of aquatic invertebrate from aquatic ecosystems in Amazon

2. Materials and methods

2.1 Test organism

The ostracod species was collected in August 2018 in the Porto Velho Natural Park, RO (8º 41’13,26” S; 63º52’10,06” W), SISBio authorization n. 67974-1, and taken to the Laboratory of Limnology and Aquatic Ecotoxicology at the Federal University of São Carlos – UFSCar. After collection, the organisms were acclimatized in the laboratory

in plastic trays with 4 L capacity and maintained at 25 °C ± 1 °C and 12 h light / 12 h dark photoperiod.

Long-term cultures were maintained in the laboratory following adaptation of the protocol for the tropical cladoceran species - *Ceriodaphnia silvestrii* (ABNT, 2022), using reconstituted water with the following parameters: pH 7-7.6, hardness 40-48 mg L^{-1} of $CaCO_3$, electrical conductivity of 160 μS cm^{-1}. The diet was based on 20 mL of Tetramin® fish food (5g solution in 1L of distilled water) and 20 mL of *Raphidocelis subcapitata* suspension (10^8 cells L^{-1}).

2.2 Acute toxicity test

The test organism, the ostracod *S. rondoniensis*, used in this study is a newly described species (Gomes et al. 2024), therefore, it is being used for the first time in ecotoxicological studies. The sensitivity range was determined following the model developed by the USEPA protocol (1985). The procedure involved the distribution of toxicity values obtained in 20 experiments with the reference substance copper sulfate and the calculation of the ratio between the upper and lower limits of the 95% confidence interval (95% CI) (Brock et al., 2008).

To evaluate the toxicity of metals, the following reagents were used: Cadmium chloride ($CdCl_2$; no. CAS 35658-65-2; Carlo Erba Reagentes®), Zinc Chloride ($ZnCl_2$; no. CAS 7646-85-7; Merck®) and Mercury Chloride ($HgCl_2$; CAS no. 7487-94-7; Merck®). Three preliminary tests were conducted to determine the range of concentrations to be used for each metal salt. A set of five concentrations was established for each metal salt (see Table S1) with concentrations ranging from 0.50 to 4.0 mg L^{-1} for $CuSO_4$, 0.1 to 0.8 mg L^{-1} for $CdCl_2$, 10 to 80 mg L^{-1} of $ZnCl_2$, and 0.1 to 1.6 for $HgCl_2$. Acute toxicity tests were carried out in 50 mL nontoxic polyethylene cups containing 20 mL of test solution, with three replicates per concentration containing five individuals each. The control treatment also included five individuals of ostracods in 20 mL, solely in reconstituted water. For each metal, five acute toxicity experiments were performed. The experiments were maintained in the dark, without feeding, under controlled temperature conditions (25 ± 1°C) and lasted 96 h. Mortality was monitored at 48, 72, and 96 h. The following physical and chemical water parameters, such as pH (Quimis Model Q400AS), electrical conductivity (EC) (Digimed DM 3), temperature (T) and dissolved oxygen concentration (DO) (Hanna Model HI9146) were measured at the

beginning and end of the experiment. The water hardness was measured only at the beginning of the tests (ABNT, 5761, 1984).

Mixture tests were carried out with binary combinations of the metal salts $CuSO_4$ and $CdCl_2$; $CuSO_4$ and $ZnCl_2$ and $CuSO_4$ and $HgCl_2$. Adult individuals of *S. rondoniensis* were exposed simultaneously in isolated tests for each metal salt and in tests with the binary mixtures, using a factorial design for toxicity with a total of 25 combinations (Table S1). The methods of exposure to the compounds and the parameters analyzed were similar to those previously described.

2.3 Stock Solutions and Chemical Analysis

Stock solutions of $CuSO_4$, $CdCl_2$, $ZnCl_2$, and $HgCl_2$ were prepared at a concentration of 10 and 1000 mg L^{-1} to carry out the experiments. The nominal concentrations of the tested substances were obtained by diluting the stock solution in reconstituted water.

To quantify the $CuSO_4$, $CdCl_2$, $ZnCl_2$ an aliquot from the stock solutions was taken, acidified with concentrated HNO_3 until pH < 2.0 and used to quantify real concentrations. These analyses were made in Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES Optima 8300/Perkin Elmer). Quantification followed recommendations from the 23rd edition of Standard Methods (SMWW, 3111 B). While the quantification of $HgCl_2$ in one sample was carried out using the DMA-80 EVO Milestone following recommendations (USEPA 7473), the quantification results are presented in Table S2.

2.4 Data analysis

To analyze the survival rates, dose-response graphs were generated in the R studio platform. The software (2023.03.1) and lethal concentrations for 50% of organisms (48/72/96h-LC_{50}) were determined by nonlinear estimations with a logistic model.

Data from mixture toxicity tests were analyzed by comparing observed data for combined effects, using concentration addition (CA) and independent action (IA) reference models in the MIXTOX tool (Jonker et al. 2005). Then, the CA and IA models were extended according to Jonker et al. (2005) and the deviation functions (synergistic or antagonistic interactions), dependent on dose ratio and dose level) were modeled by

adding the parameters "a" and "b" in a nested structure. For the synergism or antagonism deviation, parameter "a" is considered negative or positive, respectively. For the dose ratio-dependent deviation (DR), in addition to the "a" parameter, the value of the "bDR" parameter indicates that the deviation from the reference model is controlled by the composition of the mixture. For dose level-dependent deviation (DL), the parameters used are "bDL", which indicates at which dose level the deviation changes, and parameter "a", whose value indicates the deviation in high and low doses. More detailed descriptions of the functions are presented in Jonker et al. (2005). Obtained data were subjected to adjustments in conceptual models and deviation analysis and the best fit was chosen using the maximum likelihood method. For models with significant deviations, the effect pattern was deduced directly from the parameter values, and the maximum deviation was calculated in terms of the effect level (Jonker et al. 2005).

2.5 Ecological Risk Assessment

2.5.1 Species Sensitivity Distribution Curve – SSD curve

To obtain the SSD curves, acute toxicity data (LC50 and EC50) for $CuSO_4$, $CdCl_2$, $ZnCl_2$, and $HgCl_2$ were sourced for species from various trophic levels from the ECOTOX database of the U.S. EPA (https://cfpub.epa.gov/ecotox/). Furthermore, complementary information was obtained from the Web of Science, Scopus, and Science Direct repositories, by using the keywords "Ecotoxicology," "metals", "LC_{50}", and the scientific names of the aquatic species. When two or more toxicity data were obtained for the same species, the LC_{50}/EC_{50} geometric mean was used. All toxicity data and references used in the SSD curves are presented as Supplementary Material (Table S3). SSD curves, their respective Hazardous Concentrations to 5% (HC_5) and 50% (HC_{50}) of species, and 95% confidence intervals were obtained in the ETX (version 2.3) software (Van Vlaardingen et al. 2004). The Graphic representations were generated following the method described by Thorley and Schwarz (2018).

2.5.2 Determination of Risk Quotients (RQ)

The Ecological Risk Assessment - ERA was conducted using Risk Quotients (RQ), which represent the relationship between the measured environmental concentrations (Minimum, Average, and Maximum) reported in freshwater environments I the amazon (MEC water) (Table S11) and the predicted no-effect concentration for

water (PNEC water). To calculate the PNEC values, the HC_5 value (Table S4) was divided by an assessment factor (AF = 2). The choice of this AF value considered the uncertainty associated with the availability of ecotoxicological data for different taxonomic groups used in the preparation of SSD curves (EFSA, 2002). The interpretation of RQ values followed the following scale: low risk (RQ < 0.1), medium risk (RQ ranging from 0.1 to 1), and high risk (RQ > 1) (Lu et al. 2022; Yan et al. 2022; Gomes et al. 2023).

3. Results and discussion

3.1 – Water parameters of test solutions

Values of water parameters remained stable throughout the experiments. The pH ranged between 7.4-7.8, electrical conductivity 138.83-167.0 µS cm^{-1}, dissolved oxygen 5.37-6.8 mg L^{-1}, temperature 24.79-25.3 °C, and the hardness between 40-44 mg $CaCO_3$ L^{-1} (Table S5). Metal concentrations quantified in stock solutions are available in Table S2 in the supplemental material. The metal concentrations used in this study did not vary by more than 10% in relation to the desired concentrations, as recommended by the ISO standard (2000). This confirms the adequacy in using nominal concentrations.

3.2 – Sensitivity to the reference substance Copper Sulfate

In all experiments, the mortality rate in the control group remained below the recommended threshold of 10%, validating the assays. The results of the sensitivity tests are presented in Figure 1. The means and standard deviations are shown in Table S6. The data indicate an $LC_{50/48h}$ of 1.69 mg L^{-1} (IC 95%: 1.30 – 2.33 mg L^{-1}) of $CuSO_4$ for *S. rondoniensis*.

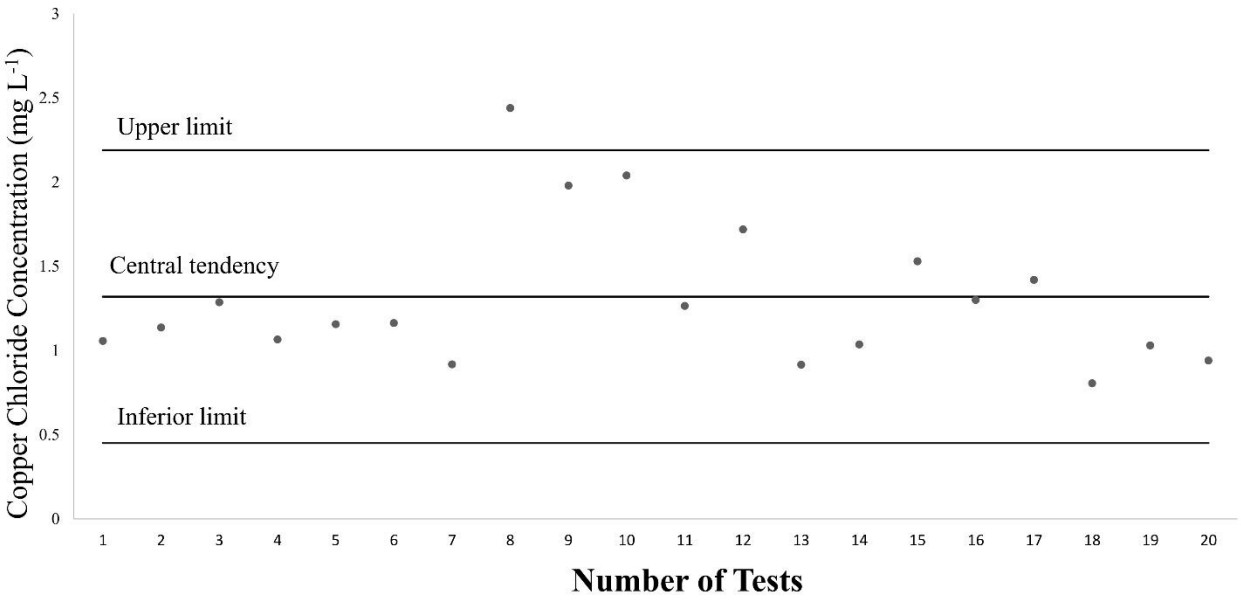


Figure 1 – Sensitivity ranges for *Strandesia rondoniensis* to copper sulfate ($CuSO_4$) based on 20 acute toxicity tests

To the best of our knowledge, the only ostracod species standardized for ecotoxicological assays is *Heterocypris incongruens* (Ramdohr, 1808), following the standard ISO-14371, (2012). This species presented a lower sensitivity to $CuSO_4$ ($LC_{50/48H}$: 5.79 mg L^{-1}) (Janssen; Persoone, 2011) compared to *S. rondoniensis.* On the other hand, when comparing the sensitivity of *S. rondoniensis* with other ostracods such as *S. trispinosa*, *Stenocypris major* and *Cypris subglobosa*, it appeared that they all have greater sensitivity to $CuSO_4$, with $LC_{50/48h}$ of 0.75; 0.038, and 0.55 mg L^{-1}, respectively (Lima et al. 2019; Shuhaimi-Othman, 2011; Khangarot, Das 2009).

3.3 - Acute toxicity of metals alone

The data of LC_{50} for 48, 72, and 96h follow a pattern already observed by other authors, in which the concentrations necessary to cause effects on the survival of ostracod individuals are lower in experiments with longer exposure times (Shuhaimi-Othman, 2011; Alberola and Joanes, 2012; Margerit et al. 2015), Table S6 and Figure 2. The results of the present study indicate that the most toxic metal to *S. rondoniensis* was Cd (48h-LC_{50}: 0.37 mg L^{-1} of $CdCl_2$), followed by the Hg ($LC_{50/48h}$: 0.95 mg L^{-1} of $HgCl_2$), Cu

($LC_{50/48h}$: 1.70 mg L^{-1} of $CuSO_4$) the Zn ($LC_{50/48h}$: 54.87 mg L^{-1} of $ZnCl_2$). According to Abel (2002), cadmium, mercury, and copper are generally the most toxic metals for aquatic organisms.

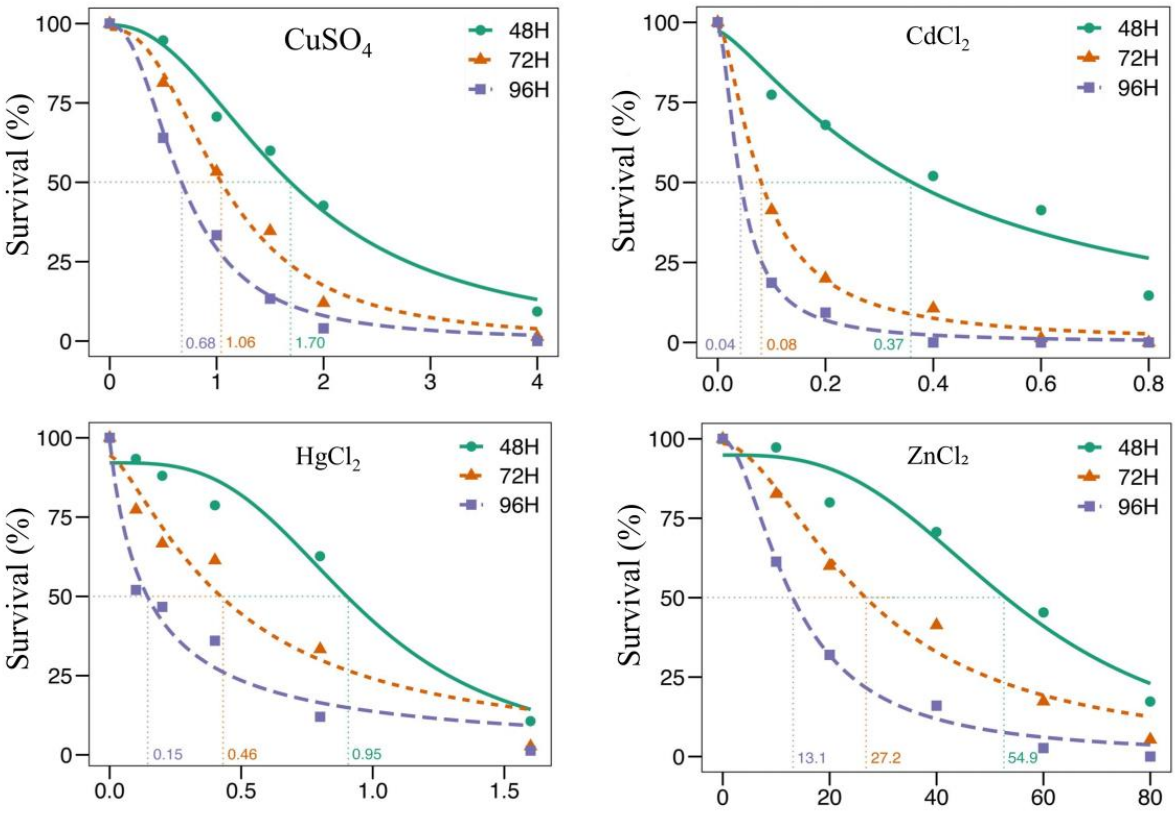


Fig 2 – Dose-response of *Strandesia rondoniensis* for acute toxicity of metals Cu, Cd, Hg, and Zn after different exposure times (48, 72 and 96h)

Cadmium is classified as a non-essential metal and highly toxic to living beings (Qian et al. 2009; Guimarães, Römbke, Amorim, 2019). High sensitivity to cadmium has been confirmed in small aquatic crustaceans as highlighted in previous studies, including Shuhaimi-Othman (2011), Alberola and Joanes (2012), and Lima et al. (2019), specifically in ostracods, as indicated in Table S7. Notably, the species *S. rondoniensis* demonstrated greater sensitivity to cadmium ($LC_{50/48h}$: 0.37 mg L^{-1} of $CdCl_2$) compared to Khangarot's data (2009), who reported an $LC_{50/48h}$ of 0.821 mg L^{-1} for the ostracod *Cypris subglobosa*. These findings have also been reported for other small crustaceans, as observed by Shuhaimi-Othman and Pascoe (2001), in which the amphipod species *Hyalella azteca* was more sensitive to cadmium than to other metals.

Furthermore, data reported by Sevilla et al. (2013, 2014) indicated greater sensitivity in individuals of *Heterocypris incongruens* when exposed to food (algae) contaminated with copper and cadmium. Carvalho et al. (2018) also observed a reduction in reproductive rates in the cladoceran *Ceriodaphnia dubia* when exposed to low concentrations of cadmium in chronic exposures. Jackson (2002) found that cadmium is more toxic to aquatic organisms when exposed to water with low hardness (20 – 130 mg $CaCO_3$ L^{-1}), similar to the conditions used in the present study. Furthermore, Kozlova, Wood, and McGeer (2009) highlighted that the toxicity of metal ions is lower in harder waters due to competition between metal and ions Ca^{2+} and Mg^{2+} at absorption sites in organisms.

The second most toxic metal to *S. rondoniensis* was Hg with an $LC_{50/48h}$ of 0.95 mg L^{-1} of $HgCl_2$. According to Ramírez-Pérez et al. (2004), reduced mercury concentrations caused a significant decrease in zooplankton survival and reproduction. However, for the ostracod *Cypris subglobosa*, mercury was the most toxic metal, presenting a $CE_{50/48H}$ from 0.369 mg L^{-1}, as reported by Khangarot and Das (2009). Data presented by Biesinger et al. (1972) also indicated adverse effects on the survival of the cladoceran *Daphnia magna* in response to low concentrations of mercury, specifically 2.7 µg L^{-1}. Studies conducted with two species of native oligochaetes, *Dero furcatus* and *Allonais inaequalis*, showed an $LC_{50/96H}$ of 0.129 and 0.092 mg L^{-1}, respectively, (Gazonato-Neto et al. 2019). It is also important to mention that, according to Xiong et al. (2022), the safe concentration of mercury for the species *Gobiocypris rarus* is below 0.0007 mg L^{-1}, as newly hatched larvae are particularly sensitive to this metal.

Zinc is the most common metal found in the Earth's crust (Ramesh, 2014). It presents high toxicity to most aquatic organisms, when exposed to high concentrations (Horie et al. 2020). In our study, it was the least toxic metal, with an $LC_{50/48H}$ from 54.87 mg L^{-1} of $ZnCl_2$ and our test species, *S. rondoniensis*, was more sensitive than the species *C. subglobosa* with one $CE_{50/48H}$ of 85.04 mg L^{-1}, (Khangarot; Das, 2009). For another species, *Stenocypris major*, Zn presented an $LC_{50/95H}$ of 168.27 mg L^{-1} (Shuhaimi-Othman, 2011), values higher than those found in the present work, for *S. rondoniensis*. On the other hand, concentrations of ≥ 0.5 mg L^{-1} of Zn were enough to cause changes in the reproduction of the cladoceran *Daphnia longispina* (Martins et al. 2017).

3.4 - Toxicity of mixtures

All binary mixtures tests performed in this study were analyzed for both the Concentration Addition (CA) model and the Independent Action (IA) model, allowing us to determine the most appropriate model for each data set.

The first combination test run in this study was using a mixture of copper sulfate and cadmium chloride. The results showed a better fit to the CA model (Table S8), with sum of squares of residuals (SS) of 59.82 ($p < 0.001$) determination coefficient of $r^2 = 0.84$. Subsequently, when adding parameters "a" and "b" to the CA model, it was found that the data fit more adequately to the Dose Ratio Dependent Deviation (DR) model due to the decrease in the SS value to 46.51, and significant correlation coefficient ($p < 0.001$), resulting in a determination coefficient of $r^2 = 0.88$. The negative values of “a” (-0.263) and “b” (-1.919) indicated synergistic effect when copper sulfate predominated in the mixture (Figure 3 and Table S8).

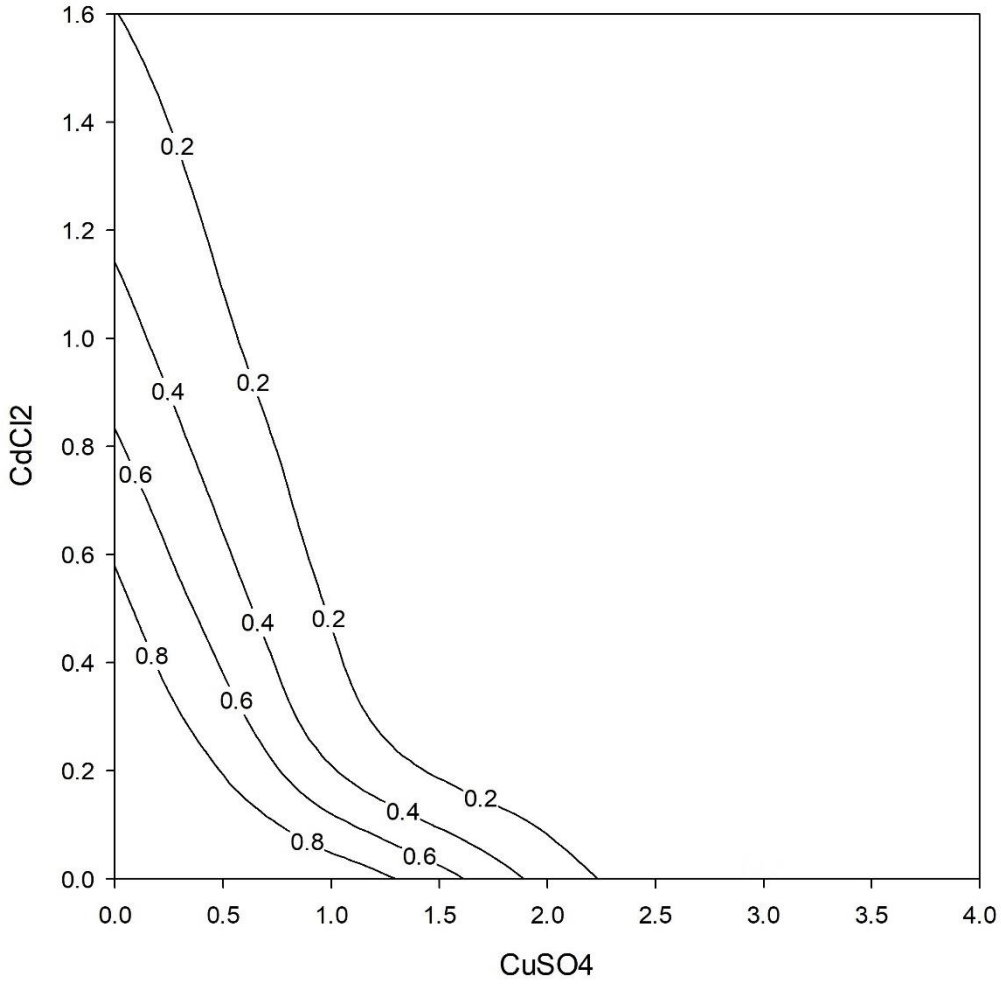


Figure 3 – Isobologram of the effects of mixtures between the metal salts $CuSO_4$ and $CdCl_2$ for the ostracod *Strandesia rondoniensis* highlighting the synergistic deviation from the AC model, for the survival results

Currently, it is widely documented in literature that synergistic effects occur between Cu and Cd concerning individual mortality of *Caenorhabditis elegans* (Chu and Chow, 2002). According to Negflski et al. (1981), an increase in toxicity was observed when exposing the shrimp *Callianassa australiensis* to different concentrations of Cu and Cd. Similarly, Qian et al. (2011) found synergistic effects in experiments with the alga *Chlorella vulgaris* marked by an increase in the amount of reactive oxygen species (ROS), the interruption of chlorophyll synthesis, and inhibition of cell growth. Studies conducted with algae of the same genus have shown synergistic effects in Cu-Cd mixtures (Franklin et al. 2002). Increases of toxicity and a significant decrease in locomotion were observed for the nematode *C. elegans* when exposed to combinations of Cu and Cd (Moyson et al. (2017). Furthermore, Martín-Folgar and Martínez-Guitarte (2019) identified changes in the Gp93 and Dronc genes, related to the immune system and apoptosis for the dipteran *Chironomus riparius*, after exposure to the same metals. They observed that these changes could compromise the larvae ability to respond to infection and the continuity of metamorphosis process.

The second combination, involving Copper and Zinc, also fitted the Concentration Addition (CA) model. This resulted in a sum of squared residuals (SS) of 74.16 ($p < 0.001$) with determination coefficient $r^2 = 0.79$. After including parameters "a" and "b" in the CA model, it was observed that the data fitted better to the Dose Ratio Dependent Deviation (DR) model. This is due to the reduction of the SS value to 31.83, together with the correlation coefficient ($p < 0.001$) and $r^2 = 0.91$ (Table S9). Obtaining the positive value for “a” (2.694) and negative value for “b” (-1.076) indicated synergism when copper was at a higher concentration and antagonism when zinc was at a higher concentration (Figure 4 and Table S9).

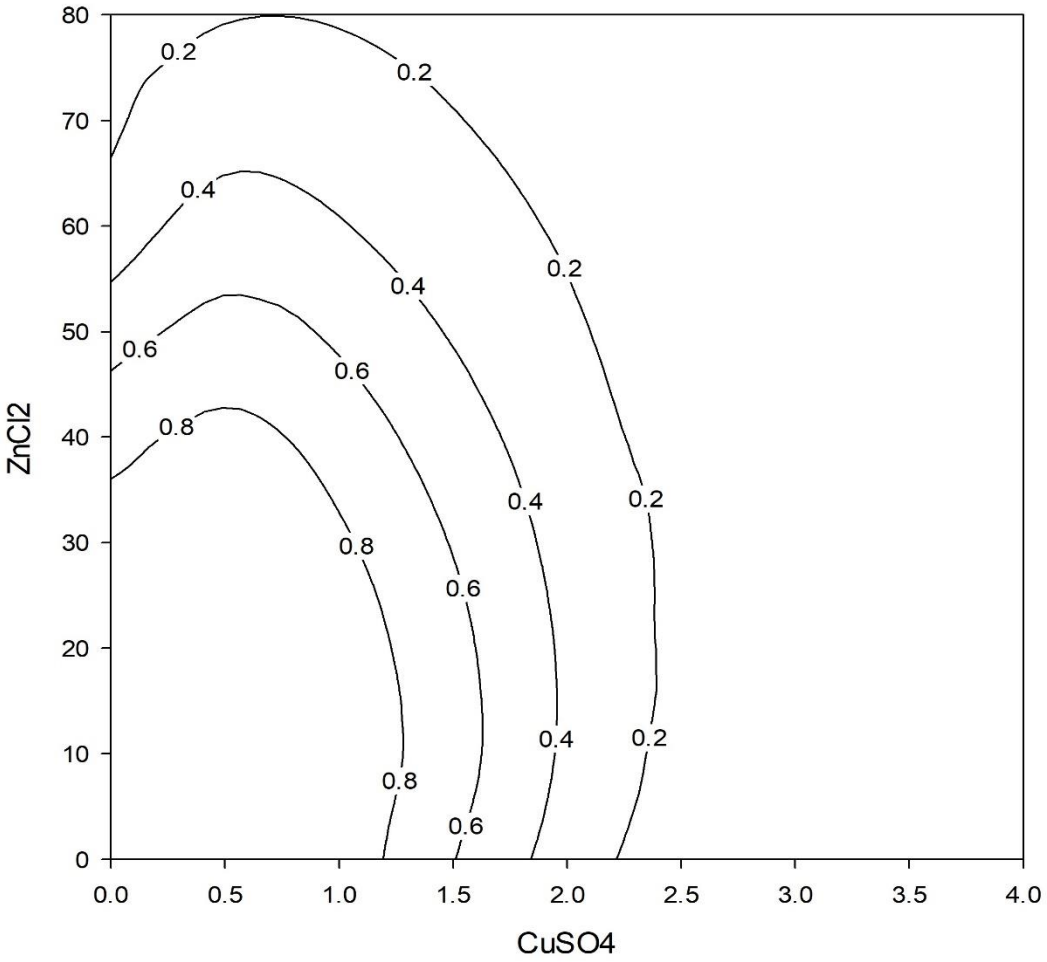


Figure 4 – Isobologram of the effects of mixtures between the metal salts $CuSO_4$ and $ZnCl_2$ for the ostracod *Strandesia rondoniensis* highlighting the synergistic and antagonistic deviation for survival results from the AC model

Optimal concentrations of essential trace elements such as copper and zinc play fundamental roles in physiological activities of a variety of aquatic invertebrates (Jeong et al. 2023) and are essential for body formation and protection against predators (Odate and Pawlik, 2007). However, they can cause toxicity at concentrations above the metabolic capacity of living organisms (Shakir et al. 2016). Synergistic effects were observed when *Caenorhabditis elegans* was exposed to several concentrations of Cu and Zn (Moyson et al. 2019). According to Jonker et al. (2005), synergistic effects between Zn and Cu were observed in the population growth of *C. elegans*, after one week of exposure at high concentrations. However, antagonistic shifts were also observed for the

diatom *Navicula pelliculosa* in combinations of Zn and Cu (Nagai and Schamphelaere, 2016). Therefore, the combination of mixture influences the responses of aquatic organisms, which may present variations in sensitivity depending on the metal.

The third combination tested, between copper sulfate and mercury chloride, better fitted the independent action (IA) model. A sum of squared residuals (SS) of 74.28 ($p = 0.001$) was generated, with an r^2 of 0.79. However, after adding parameters "a" and "b" to the IA model, data fit the dose ratio-dependent deviation (DR) model better due to the decrease in the SS value to 39.16 correlation ($p < 0.001$) and determination ($r^2 = 0.89$) coefficients values obtained (Table S10). The positive value of “a” (3.667) and the negative value of “b” (-17.336) indicated synergism when copper was at a higher concentration and antagonism when mercury was higher. The dose-response pattern for this mixture is presented in Figure 5 and Table S10.

The results for the interaction between Cu and Hg on *S. rondoniensis* are in line with findings from Lima et al. (2023). These authors observed synergistic effects when copper was at a higher concentration in the mixture for the ostracod *S. trispinosa*, while antagonism was found when mercury predominated. An antagonistic effect between copper and mercury was observed in sublethal exposures to *Euplotes crassus* in a prolonged study by Gomiero et al. (2012). Furthermore, a study identified that the mixture of Hg and Cu led to antagonistic effects on oyster larvae *Crassostrea gigas* (Araújo and Silva, 1998). These findings highlight the occurrence of complex interactions between metals in freshwater and marine organisms, emphasizing the importance of detailed ecotoxicological studies to understand the effects of different substances in aquatic ecosystems. Under certain circumstances, the co-occurrence of different metals can significantly increase the toxicity of each isolated pollutant (Gomiero et al. 2012), as observed in some interactions in this study.

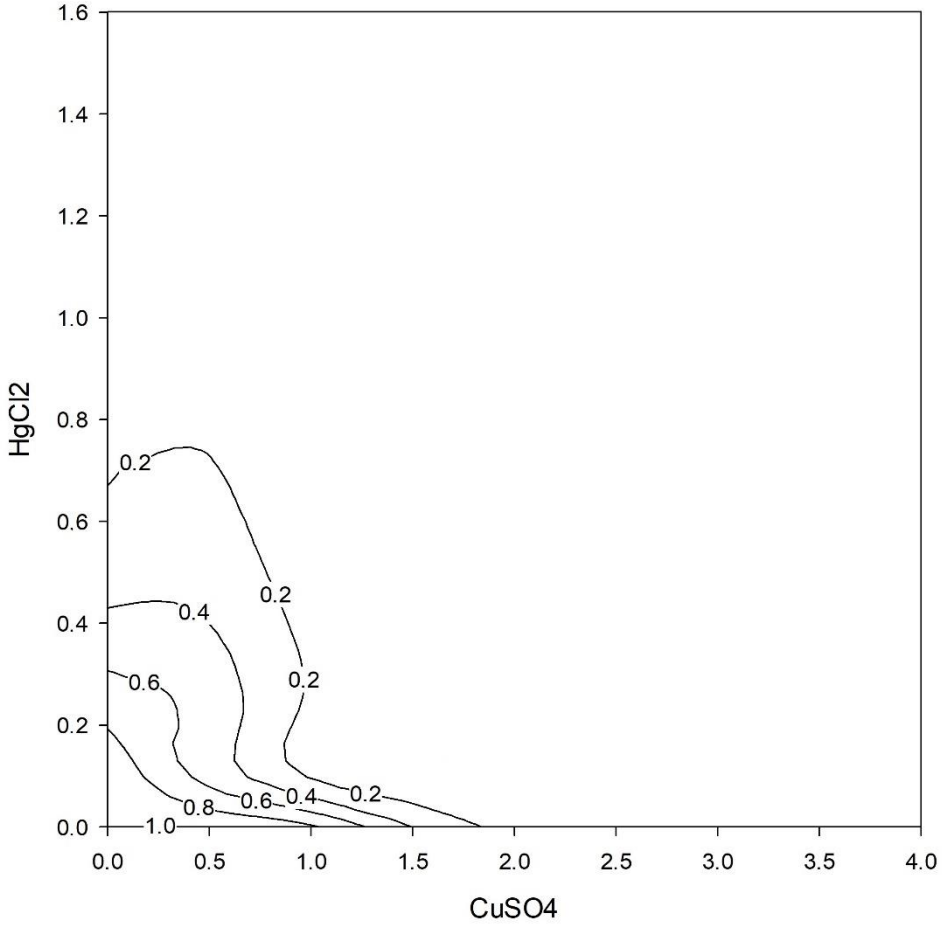


Figure 5 – Isobologram of the effects of mixtures between the metal salts $CuSO_4$ and $HgCl_2$ for the ostracod *Strandesia rondoniensis* highlighting the antagonistic and synergistic deviation for survival results from the AI model

3.5 – Water Ecological Risk Assessment

The results from the risk assessment showed that 66% of all simulations for the metal concentrations tested in the present study represented a high risk (RQ ≥ 1) for Cu (Min, Med, Max) – RQ: 0.07; 8.05; 82.12, Zn (Min, Med, Max) – RQ: 0.01; 0.16; 0.72. Hg (Min, Med, Max) – RQ: 0.02; 0.03; 20. Cd (Min, Med, Max) – RQ: 1.20; 864.00; 3064.00. The HC5 and HC50 values and the sensitivity curves are presented in Table S11 and Figure 6. Our results confirm the high risk, which is in line with those pointed out by Gomes et al. (2023), who performed a risk assessment for different metals in water in the Amazon region of Brazil. These authors observed a high risk for the following metals:

Cd, Cu, Cr, and Co. Exploration of minerals in the Amazon has been correlated to the emission and propagation of metals and metalloids, as reported by Galarza et al. (2022). This fact represents a threat to human health and the integrity of the ecosystems. The contamination rates of water and sediments by carcinogenic and non-carcinogenic substances exceed established limit parameters, indicating a significant risk for local human communities dependent on natural resources such as water, vegetables, and fish for their subsistence (Galarza et al. 2022). Cd is a highly persistent metal, remaining in aquatic organisms for long periods due to the bioaccumulation process (Gestin et al. 2022). Effects of Cd, including the decrease in reproductive output and length of *D. magna* were observed at concentrations varying from 1 and 10 μg L^{-1} Cd (Na et al. 2021). Ren et al. (2019) in turn, highlighted that the activities of superoxide dismutase (SOD), glutathione S-transferase (GST), Metallothionein (MT), and DNA strand breakage were increased in the shrimp *Marsupenaeus japonicus* when exposed to concentrations of 5, 50 and 500 μg L^{-1} of Cd.

Copper can trigger a series of adverse effects in aquatic invertebrates, such as induction of oxidative stress, changes in enzyme function, inhibition of growth and reproduction, disruption of the endocrine system, and reduction in the capacity to acquire energy (Perić et al. 2020; Le et al. 2021). Recently, Morroni et al. (2023) found that exposure to Cu, at (concentrations ranging from 10-40 μg L^{-1}) increased abnormal embryo formation for the black sea urchin *Arbacia lixula.* Chain et al. (2019) observed that the species *Daphnia pulex* had an increase in the expression of MT, GST, and metal carboxypeptidase when exposed to 90 μg L^{-1} of Cu. In the freshwater cnidarian *Hydra magnipapillata* exposed to copper concentrations between 0.06 and 0.1 mg L^{-1}, reactive oxygen species (ROS) were detected, and the expression of genes associated with antioxidant activity—catalase (CAT), SOD, glutathione peroxidase (GPx), GST, glutathione reductase (GR)—increased. Moreover, DNA damage was observed (Zeeshan et al. 2016).

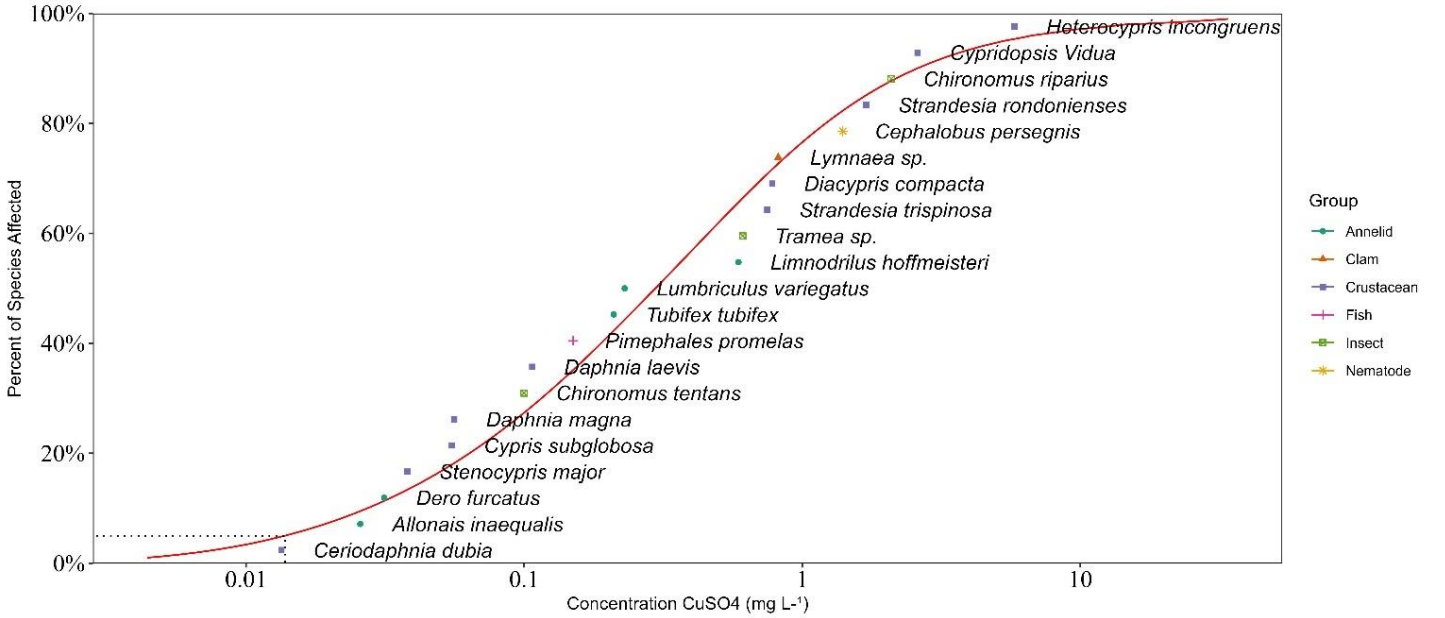
For decades, several studies have reported that the Amazon is heavily contaminated with Hg (Pfeiffer and Lacerda, 1988; Lodenius and Malm, 1998), whether it enters naturally (Fadini and Jardim, 2001), or through mining activities that have persisted for decades in the region up to the present day (Veiga et al. 2002; Fritz et al. 2023). Exposure to Hg caused an increase in GST activity in the cladoceran *Diaphanosoma celebensis* (Yoo et al. 2022). Melo et al. (2021) reported that genes related to antioxidant enzymes, defense-linked antioxidants, endocrine system genes, and the

immune system were affected, while genes related to respiration and apoptosis were expressed at high levels. It is known that mercury has a significant affinity for the sulfide group (-SH) and quickly binds to cysteines found in proteins, resulting in functional impairments and toxicity in aquatic invertebrates (Xu et al. 2016).

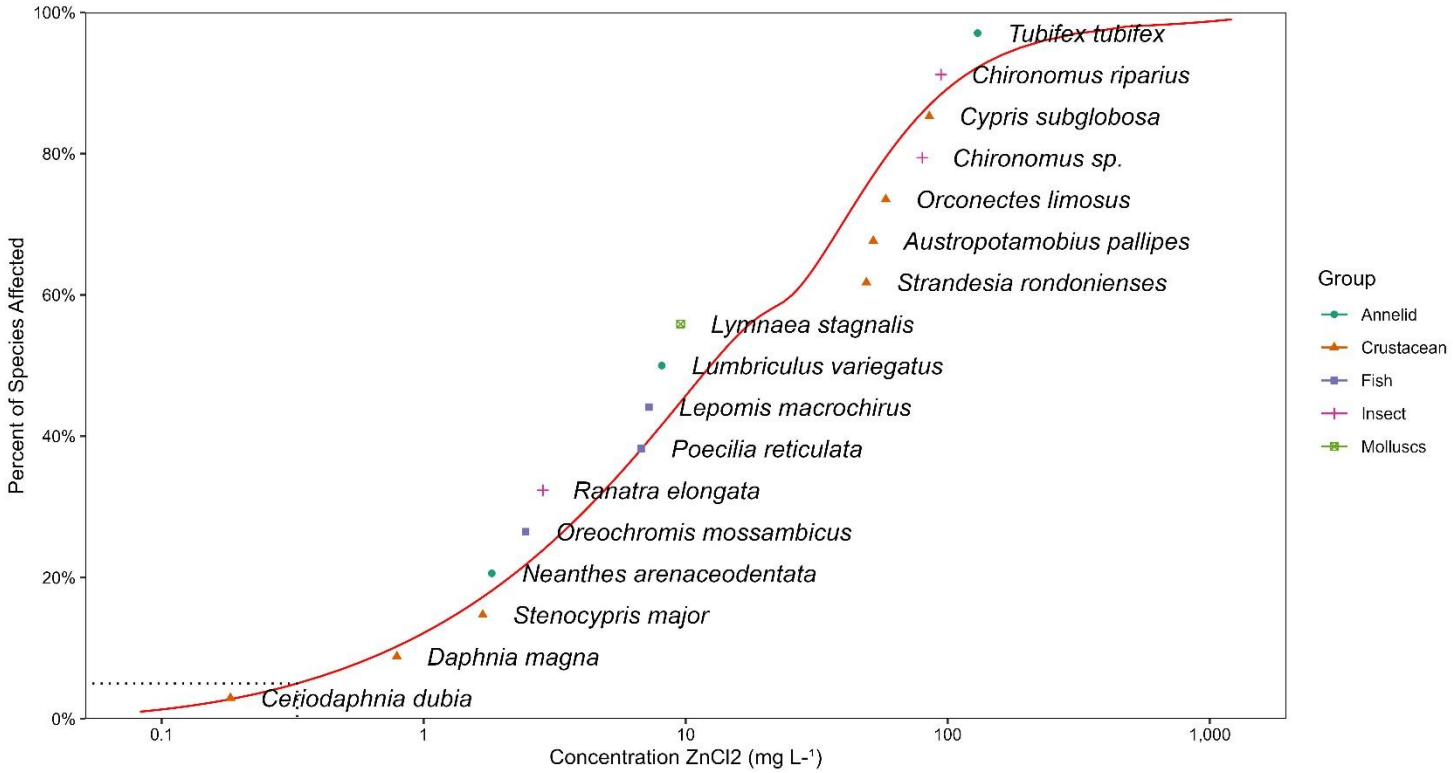
Although zinc is an essential metal for living beings, it can be toxic if present in high concentrations (Horie et al. 2020). However, Liang et al. (2022) found that Zn concentrations between 0.01 and 1 mg L^{-1} caused a decrease in growth and survival rates of the shrimp *Litopenaeus vannamei,* in addition to structural damage. Zinc accumulation attenuated total antioxidant capacity and increased ROS. Lee et al. (2021) in turn, conducted studies with *D. magna* at concentrations between 0.75 and 1.5 mg L^{-1} and concluded that there was a decrease in fertility and an increase in oxidative stress. Wang et al. (2020) used the mollusk *Lampsilis siliquoidea* as a test organism and verified that Zn concentrations between 15 and 240 µg L^{-1} caused decrease in biomass, dry weight, and body length of the organisms.

Currently, the extensive scientific literature offers significant evidence of environmental changes resulting from human interventions in the Amazon region. According to Albert et al. (2022), human activities such as deforestation, forest fires, damming and fragmentation of the river basin have directly contributed to this process, on a hitherto unprecedented scale. Lapola et al. (2023) point out that an area of approximately 2.5 million square kilometers of the Amazon Forest has been degraded due to fires in recent years. Moreover, the increase in mining (legal and illegal) shows that this activity play a significant role in the entry of metals into aquatic ecosystems of the Amazon.

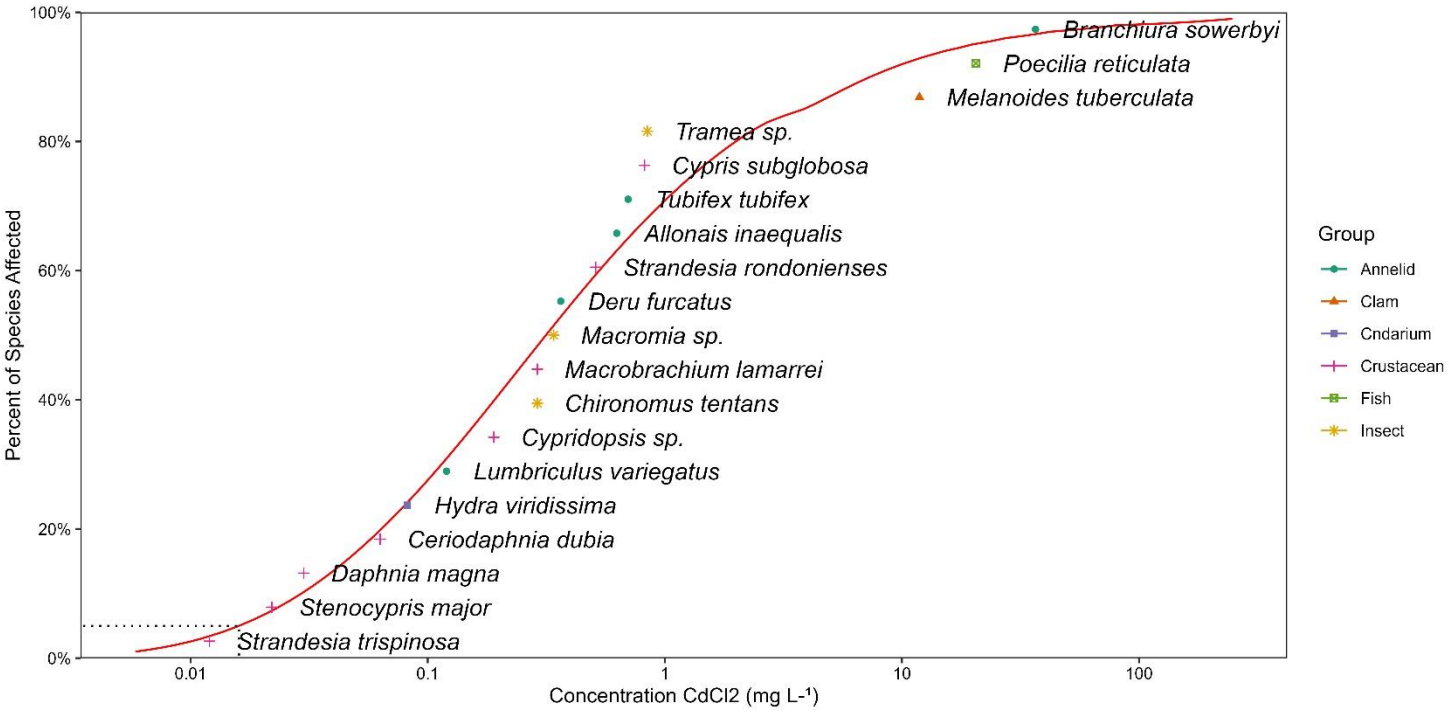
A


100%
80%
60%
40%
20%
0%
Percent of Species Affected
0.01
0.1
1
10
Concentration CuSO4 (mg L-1)
Heterocypris incongruens
Cypridopsis Vidua
Chironomus riparius
Strandesia rondonienses
Cephalobus persegnis
Lymnaea sp.
Diacypris compacta
Strandesia trispinosa
Tramea sp.
Limnodrilus hoffmeisteri
Lumbriculus variegatus
Tubifex tubifex
Pimephales promelas
Daphnia laevis
Chironomus tentans
Daphnia magna
Cypris subglobosa
Stenocypris major
Dero furcatus
Allonais inaequalis
Ceriodaphnia dubia
Group
Annelid
Clam
Crustacean
Fish
Insect
Nematode

B


100%
80%
60%
40%
20%
0%
Percent of Species Affected
0.1
1
10
100
1,000
Concentration ZnCl2 (mg L-1)
Tubifex tubifex
Chironomus riparius
Cypris subglobosa
Chironomus sp.
Orconectes limosus
Austropotamobius pallipes
Strandesia rondonienses
Lymnaea stagnalis
Lumbriculus variegatus
Lepomis macrochirus
Poecilia reticulata
Ranatra elongata
Oreochromis mossambicus
Neanthes arenaceodentata
Stenocypris major
Daphnia magna
Ceriodaphnia dubia
Group
Annelid
Crustacean
Fish
Insect
Molluscs

C



D

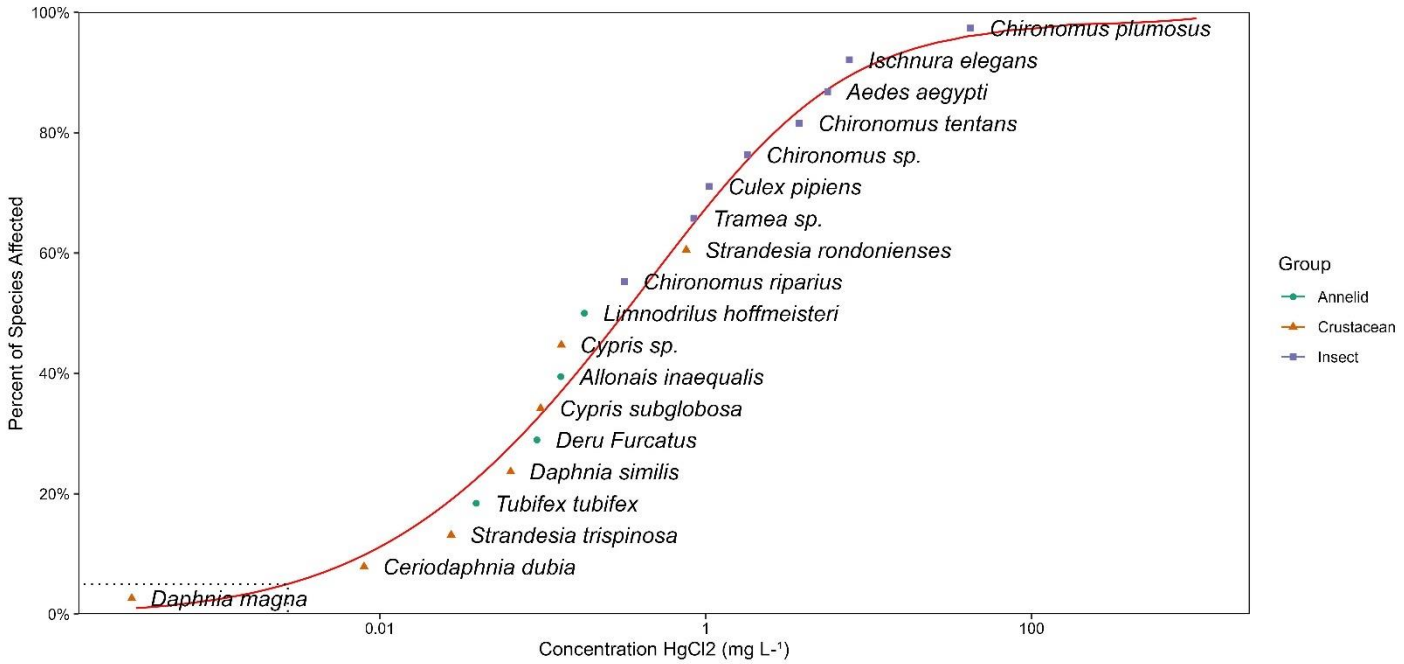


Figure 6 – Species sensitivity distributions (SSD) constructed based on LC_{50} values (geometric mean) for Cu (A), Zn (B) Cd (C), and Hg (D) obtained in the present study for *Strandesia rondoniensis* and literature for other aquatic organisms

Results from metal pollution consequences summarized above may be representative of the effects of the high concentrations of metals present in the Amazon aquatic ecosystems (Gomes et al. 2023). Viana et al. (2020) quantified concentrations in the Araguari River, finding Cd = 0.009 mg L^{-1}; Cu = 0.011; Hg = 0.009; Zn = 4.640 mg L^{-1}. In turn, Pinto et al. (2009) reported relatively high values in studies carried out in the Negro and Amazonas rivers reaching 0.18 mg L^{-1} for Cd, 0.22 mg L^{-1} for Cu, and 0.04 mg L^{-1} for Zn. Meanwhile, Santana and Barroncas (2007) found concentrations of Cu, Zn in the Tarumã-Açu basin reaching 0.10 and 0.21 mg L^{-1}, respectively. Lima et al. (2015) recorded values of Cd – 0.164, Cu – 0.37, and Zn – 0.139 mg L^{-1}, in the Cassiporé River, Amapá (also in the Amazonian region).

As seen in Table S4, in many cases, environmental concentrations significantly exceed HC5 or even HC50 values, which reinforces the elevated risk for aquatic species in Amazonian ecosystems. Therefore, it is essential to urgently reestablish public policies focused on continuous monitoring of the quality of water bodies in the Amazon region to reduce the impacts generated by human activities. This is fundamental for the conservation and protection of the whole aquatic Amazonian ecosystems.

4. Conclusion

The results of this study show that the sensitivity of the ostracod *S. rondoniensis* to metals was high and close to the values reported for other ostracod species. Cadmium stood out as the most toxic metal tested, followed by Hg, Cu, and Zn. Furthermore, an increase in the toxicity of these metals was observed as the exposure time increased. In metal mixtures, in turn, different effects were identified, with predominance of synergism when copper was present in high concentrations. Moreover, the risk assessment pointed to a high ecological risk ($RQ \geq 1$) concerning tested metals (Cd; Cu; Hg). Therefore, the continued contamination of aquatic ecosystems in the Amazon region represents a worrying threat to aquatic biota, since the concentrations found in our study are considerably above the tolerance limits of many species, especially for the ostracod *S. rondoniensis* used in this study.

References

Abel, P. D. (2002). *Water pollution biology*. CRC Press.

ABNT - Associacão Brasileira de Normas Técnicas, 2022. NBR 13373. Aquatic Ecotoxicology - Chronic Toxicity - Test with *Ceriodaphnia* spp. (Crustacea, Cladocera), Rio de Janeiro, Brazil.

Aguilar-Alberola, J. A., & Mesquita-Joanes, F. (2012). Acute toxicity tests with cadmium, lead, sodium dodecyl sulfate, and *Bacillus thuringiensis* on a temporary pond ostracod. *International review of hydrobiology*, *97*(4), 375-388.

Albert, J. S., Carnaval, A. C., Flantua, S. G., Lohmann, L. G., Ribas, C. C., Riff, D., Nobre, C. A. (2023). Human impacts outpace natural processes in the Amazon. *Science*, *379*(6630).

Ali, H., Khan, E., Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry*, *2019*.

Altenburger, R.; Backhaus, T.; Boedeker, W.; Faust, M.; Scholze, M. Simplifying complexity: mixture toxicity assessment in the last 20 years. Environmental Toxicology and Chemistry, v. 32, p. 1685-1687, 2013.

Araujo, M., Silva, M. A. (1998). The Combined Effects Of Temperature And Metals Copper, Zinc And Mercury On The Embryological Development Of The Mangrove Oyster, *Crassostrea rhizophorae*. *Restoration*, *1*(1), 21-32.

Araujo, M., Silva, M. A. (1998). The Combined Effects Of Temperature And Metals Copper, Zinc And Mercury On The Embryological Development Of The Mangrove Oyster, Crassostrea Rhizophorae. *Restoration*, *1*(1), 21-32.

Araújo, S. N., Ramos, S. J., Martins, G. C., Teixeira, R. A., de Souza, E. S., Sahoo, P. K., Dall'Agnol, R. (2022). Copper mining in the eastern Amazon: an environmental perspective on potentially toxic elements. *Environmental Geochemistry and Health*, 1-15.

Arreguin-Rebolledo, U., Gebara, R. C., Gladys, V. C., Roberto, R. M., Gabriel, F. E. M., Elson, L., Federico, P. O. (2024). Toxicity of binary-metal mixtures (As, Cd, Cu, Fe, Hg,

Pb and Zn) in the euryhaline rotifer *Proales similis*: Antagonistic and synergistic effects. *Marine Pollution Bulletin*, *198*, 115819.

Atchison, G. J., Henry, M. G., Sandheinrich, M. B. (1987). Effects of metals on fish behavior: a review. *Environmental Biology of fishes*, *18*, 11-25.

Bertoletti, E. (2009). Sensibilidade de algumas espécies de peixes de água doce utilizadas no Brasil. *J. Braz. Soc. Ecotoxicol*, *4*(1-3), 9-13.

Biesinger, K. E., Christensen, G. M. (1972). Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. *Journal of the Fisheries Board of Canada*, *29*(12), 1691-1700.

BRASIL - CONSELHO NACIONAL DO MEIO AMBIENTE (CONAMA). Resolução nº 357, de 17 de março de 2005. Ministério do Meio Ambiente.

Brock, T. C., Solomon, K. R., Van Wijngaarden, R. P. A., Maltby, L. (2008). Temporal extrapolation in ecological effect assessment of chemicals. *Extrapolation practice for ecotoxicological effect characterization of chemicals. SETAC Press & CRC Press, Boca Raton*, 187-221.

Cabrera, M., Capparelli, M. V., Ñacato-Ch, C., Moulatlet, G. M., López-Heras, I., González, M. D., Rico, A. (2023). Effects of intensive agriculture and urbanization on water quality and pesticide risks in freshwater ecosystems of the Ecuadorian Amazon. *Chemosphere*, 139286.

Capparelli, M.V., Moulatlet, G.M., Abessa, D.M.S., Lucas-Solis, O., Rosero, B., Galarza, E., Tuba, D., Carpintero, N., Ochoa-Herrera, V., Cipriani-Avila, I., (2020). An integrative approach to identify the impacts of multiple metal contamination sources on the Eastern Andean foothills of the Ecuadorian Amazonia. *Sci. Total Environ*. 709, 136088.

Carvalho, M. M., Lira, V. S., Watanabe, C. H., Fracácio, R. (2017). Estudo da toxicidade de metais (zinco e cádmio) sobre *Ceriodaphnia dubia*, por multivias de exposição e recuperação biológica de descendentes. *Engenharia Sanitária e Ambiental*, *22*, 961-968.

Chain, F. J., Finlayson, S., Crease, T., Cristescu, M. (2019). Variation in transcriptional responses to copper exposure across *Daphnia pulex* lineages. *Aquatic Toxicology*, *210*, 85-97.

Chen, L., Huo, Z., Su, C., Liu, Y., Huang, W., Liu, S., Sui, Q. (2022). Sensitivity of Ostracods to U, Cd and Cu: The Case of *Cypridopsis vidua*. *Toxics*, *10*(7), 349.

Chiarelli, R., Roccheri, M. C. (2014). Marine invertebrates as bioindicators of heavy metal pollution. *Open Journal of Metal*, 4, 93-106.

Chu, K. W., Chow, K. L. (2002). Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivative. *Aquatic Toxicology*, *61*(1-2), 53-64.

Coviaga, C., Cusminsky, G., Baccalá, N., Pérez, A. P. (2015). Dynamics of ostracod populations from shallow lakes of Patagonia: life history insights. *Journal of Natural History*, *49*(17-18), 1023-1045.

Crespo-Lopez, ME, Arrifano, GP, Augusto-Oliveira, M., Macchi, BM, Lima, RR, do Nascimento, JLM, Souza, CB (2023). Mercúrio na Amazônia: O perigo de uma história única. *Ecotoxicologia e Segurança Ambiental*, *256*, 114895.

Daam, M. A., Van den Brink, P. J. (2010). Implications of differences between temperate and tropical freshwater ecosystems for the ecological risk assessment of pesticides. *Ecotoxicology*, *19*, 24-37.

dos Santos Lima, J. C., Moreira, R. A., Neto, A. J. G., Freitas, E. C., Daam, M. A., Rocha, O. (2023). Toxicity of Binary Metal Mixtures to the Tropical Ostracod *Strandesia trispinosa*. *Bulletin of Environmental Contamination and Toxicology*, *110* (3), 64.

dos Santos Lima, J. C., Neto, A. J. G., de Pádua Andrade, D., Freitas, E. C., Moreira, R. A., Miguel, M., Rocha, O. (2019). 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*, *180*, 535-541.

Ebrahimpour, M., Alipour, H., Rakhshah, S. (2010). Influence of water hardness on acute toxicity of copper and zinc on fish. *Toxicology and Industrial Health*, *26*(6), 361-365.

EFSA. European Food Safety Authority, 2002. Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances. EFSA supporting publication: EN-784. 39 p.

Elnabi, M. K., Elkaliny, N. E., Elyazied, M. M., Azab, S. H., Elkhalifa, S. A., Elmasry, S., Mahmoud, Y. A. G. (2023). Toxicity of heavy metals and recent advances in their removal: A review. *Toxics*, *11*(7), 580.

Epa, U. (1998). Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectropho-tometry. *Washington, DC: Environmental Protection Agency*.

Fadini, P. S., Jardim, W. F. (2001). Is the Negro River Basin (Amazon) impacted by naturally occurring mercury?. *Science of the Total Environment*, *275*(1-3), 71-82.

Felipe, M. C., Bernegossi, A. C., Castro, G. B., Pinheiro, F. R., Nadai, B. L. D., Cardoso-Silva, B. N., Corbi, J. J. (2020). The use of an *Allonais inaequalis* reproduction test as an ecotoxicological bioassay. *Ecotoxicology*, *29*, 634-638.

Fernández, N., Beiras, R. (2001). Combined toxicity of dissolved mercury with copper, lead and cadmium on embryogenesis and early larval growth of the *Paracentrotus lividus* sea-urchin. *Ecotoxicology*, *10*, 263-271.

Freitas, E. C., Rocha, O. (2014). Acute and chronic toxicity of chromium and cadmium to the tropical cladoceran *Pseudosida ramosa* and the implications for ecotoxicological studies. *Environmental Toxicology*, *29*(2), 176-186.

Fritz, B., Peregovich, B., da Silva Tenório, L., da Silva Alves, A. C., Schmidt, M. (2023). Mercury and CO2 emissions from artisanal gold mining in Brazilian Amazon rainforest. *Nature Sustainability*, 1-8.

Gaetke, L. M., Chow, C. K. (2003). Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology*, *189*(1-2), 147-163.

Galarza, E., Moulatlet, G. M., Rico, A., Cabrera, M., Pinos-Velez, V., Pérez-González, A., Capparelli, M. V. (2023). Human health risk assessment of metals and metalloids in mining areas of the Northeast Andean foothills of the Ecuadorian Amazon. *Integrated Environmental Assessment and Management*, *19*(3), 706-716.

Gardham, S., Hose, G. C., Stephenson, S., Chariton, A. A. (2014). DNA metabarcoding meets experimental ecotoxicology: Advancing knowledge on the ecological effects of copper in freshwater ecosystems. In *Advances in ecological research* (Vol. 51, pp. 79-104). Academic Press.

Gestin, O., Lopes, C., Delorme, N., Garnero, L., Geffard, O., Lacoue-Labarthe, T. (2022). Organ-specific accumulation of cadmium and zinc in Gammarus fossarum exposed to environmentally relevant metal concentrations. *Environmental Pollution*, *308*, 119625.

Gomes, D. F. Pereira J.S., Pinto, R.L., Moreira, R. Ap., Rocha, O., (2024). On a new species of ostracod from the Brazilian Amazon and its potential for experimental studies in laboratory culture. submitted to "Limnologica".

Gomes, D. F., da Silva Pinto, T. J., Raymundo, L. B., da Fontoura Sperandei, V., Daam, M., Moreira, R. A., Rocha, O. (2023). Ecological risk assessment for metals in sediment and waters from the Brazilian Amazon region. *Chemosphere*, *345*, 140413.

Gomiero, A., Sforzini, S., Dagnino, A., Nasci, C., Viarengo, A. (2012). The use of multiple endpoints to assess cellular responses to environmental contaminants in the interstitial marine ciliate Euplotes crassus. *Aquatic toxicology*, *114*, 206-216.

Guimarães, B., Römbke, J., Amorim, M. J. B. (2019). Novel egg life-stage test with Folsomia candida–A case study with Cadmium (Cd). *Science of the total environment*, *647*, 121-126.

Gunderson, M. P., Boyd, H. M., Kelly, C. I., Lete, I. R., McLaughlin, Q. R. (2021). Modulation of endogenous antioxidants by zinc and copper in signal crayfish (*Pacifastacus leniusculus*). *Chemosphere*, *275*, 129982.

Hautman, D. P., & Munch, D. J. (1997). Method 300.1 Determination of inorganic anions in drinking water by ion chromatography. *EPA: Ohio*.

Havel, J. E., Talbott, B. L. (1995). Life history characteristics of the freshwater ostracod *Cyprinotus incongruens* and their application to toxicity testing. *Ecotoxicology*, *4*, 206-218.

Horie, Y.; Yonekura, K.; Suzuki, A.; Takahashi, C. Zinc chloride influences embryonic development, growth, and Gh/Igf-1 gene expression during the early life stage in zebrafish (*Danio rerio*). *Comp Biochem Physiol C Toxicol Pharmacol*, 230, p. 108684, 2020

Horne, D. J., Cohen, A., Martens, K. (2002). Taxonomy, morphology and biology of Quaternary and living Ostracoda. *Washington DC American Geophysical Union Geophysical Monograph Series*, *131*, 5-36.

ISO - International Standard Organization (2000) Water quality – determination of long-term toxicity of substances to *Daphnia magna* Straus. ISO 10706, Paris

ISO, 2012. Water Quality — Determination of Freshwater Sediment Toxicity to *Heterocypris incongruens* (Crustacea, Ostracoda), first ed. The International Organization for Standardization, Geneva, Switzerland, pp. 14371 (ISSO 14371:2012(E). J. Barlow, E. Berenguer, R. Carmenta, F. França, (2020) Clarifying Amazonia's burning crisis. Glob. Chang. Biol. 26, 319–321.

JACKSON, J. (2002). Metais pesados e outras substâncias tóxicas inorgânicas. *Gerenciamento de Substâncias Tóxicas em lagos e reservatórios. São Carlos: ILEC*, 71-87.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscip. *Toxicol*. 7, 60–72.

Janssen, C. R., Persoone, G. (2011). International interlaboratory comparison on the subchronic toxicity test with the freshwater ostracod crustacean *Heterocypris incongruens*. *Report in www. microbiotests. be, section "Publications and Reports*, 1-16.

Jeong, H., Byeon, E., Kim, D. H., Maszczyk, P., Lee, J. S. (2023). Heavy metals and metalloid in aquatic invertebrates: A review of single/mixed forms, combination with other pollutants, and environmental factors. *Marine Pollution Bulletin, 191*, 114959.

Jonker, M. J., Piskiewicz, A. M., Castellà, N. I. I., Kammenga, J. E. (2005). Toxicity of binary mixtures of cadmium-copper and carbendazim-copper to the nematode *Caenorhabditis elegans*. *Environmental Toxicology and Chemistry: An International Journal*, *23*(6), 1529-1537.

Khangarot, B. S.; DAS, S. Acute toxicity of metals and reference toxicants to a freshwater ostracod, *Cypris subglobosa* Sowerby, 1840 and correlation to EC50 values of other test models. *Journal of Hazardous Materials*, v. 172, n. 2-3, p. 641-649, 2009.

Koppel, D. J., Adams, M. S., King, C. K., Jolley, D. F. (2018). Chronic toxicity of an environmentally relevant and equitoxic ratio of five metals to two Antarctic marine microalgae shows complex mixture interactivity. *Environmental Pollution*, *242*, 1319-1330.

Kozlova, T., Wood, C. M., McGeer, J. C. (2009). The effect of water chemistry on the acute toxicity of nickel to the cladoceran *Daphnia pulex* and the development of a biotic ligand model. *Aquatic Toxicology*, *91*(3), 221-228.

Lapola, D. M., Pinho, P., Barlow, J., Aragão, L. E., Berenguer, E., Carmenta, R., Walker, W. S. (2023). The drivers and impacts of Amazon forest degradation. *Science*, *379*(6630), eabp8622.

Lee, Y., Yoon, D. S., Lee, Y. H., Kwak, J. I., An, Y. J., Lee, J. S., Park, J. C. (2021). Combined exposure to microplastics and zinc produces sex-specific responses in the water flea *Daphnia magna*. *Journal of Hazardous Materials*, *420*, 126652.

Li, M., Liu, W., Slaveykova, V. I. (2020). Effects of mixtures of engineered nanoparticles and metallic pollutants on aquatic organisms. *Environments*, *7*(4), 27.

Liang, Z., Chen, T., Yang, F., Li, S., Zhang, S., & Guo, H. (2022). Toxicity of chronic waterborne zinc exposure in the hepatopancreas of white shrimp *Litopenaeus vannamei*. *Chemosphere*, *309*, 136553.

Lodenius, M., & Malm, O. (1998). Mercury in the Amazon. *Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews*, 25-52.

Lu, J., Wang, S., Li, Y., F., 2022. Ecological risk assessment of ammonia nitrogen in the rare fish reserve in the upper reaches of the Yangtze River. *Water resources*, 40, 53–56.

Margerit, A., Lecomte-Pradines, C., Svendsen, C., Frelon, S., Gomez, E., Gilbin, R. (2015). Nested interactions in the combined toxicity of uranium and cadmium to the nematode *Caenorhabditis elegans*. *Ecotoxicology and environmental safety*, *118*, 139-148.

Mariani, L., Grenni, P., Caracciolo, A. B., Pescatore, T., Spataro, F., Rauseo, J., Patrolecco, L. (2022). Use of the *Heterocypris incongruens* bioassay for assessing ecotoxicity of soils containing the anionic surfactant sodium lauryl ether sulphate (SLES). *Ecological Indicators*, *145*, 109597.

Martín-Folgar, R., Martínez-Guitarte, J. L. (2019). Effects of single and mixture exposure of cadmium and copper in apoptosis and immune related genes at transcriptional level on the midge *Chironomus riparius* Meigen (Diptera, Chironomidae). *Science of the Total Environment*, *677*, 590-598.

Martins, C., Jesus, F. T., Nogueira, A. J. (2017). The effects of Copper and Zinc on survival, growth and reproduction of the cladoceran *Daphnia longispina*: introducing new data in an "old" issue. *Ecotoxicology*, *26*, 1157-1169.

Martins, R. T., Couceiro, S. R., Melo, A. S., Moreira, M. P., Hamada, N. (2017). Effects of urbanization on stream benthic invertebrate communities in Central Amazon. *Ecological indicators*, *73*, 480-491.

McKinley, K., McLellan, I., Gagné, F., Quinn, B. (2019). The toxicity of potentially toxic elements (Cu, Fe, Mn, Zn and Ni) to the cnidarian *Hydra attenuata* at environmentally relevant concentrations. *Science of the Total Environment*, *665*, 848-854.

Melo, M. S., Das, K., Gismondi, E. (2021). Inorganic mercury effects on biomarker gene expressions of a freshwater amphipod at two temperatures. *Ecotoxicology and Environmental Safety*, *209*, 111815.

Meng, Q., Li, X., Feng, Q., Cao, Z. (2008). The acute and chronic toxicity of five heavy metals on the Daphnia magna. In *2008 2nd International Conference on Bioinformatics and Biomedical Engineering* (pp. 4555-4558). IEEE.

Morroni, L., Gaion, A., Broccoli, A., Ferrari, S., Pellegrini, D., Sartori, D. (2023). Influence of Salinity on Copper Toxicity in *Paracentrotus lividus* and *Arbacia lixula* Embryos. *Water*, *15*(1), 65.

Moulatlet, G. M., Yacelga, N., Rico, A., Mora, A., Hauser-Davis, R. A., Cabrera, M., Capparelli, M. V. (2023). A systematic review on metal contamination due to mining activities in the Amazon basin and associated environmental hazards. *Chemosphere*, 139700.

Moyson, S., Town, R. M., Vissenberg, K., Blust, R. (2019). The effect of metal mixture composition on toxicity to C. elegans at individual and population levels. *PLoS One*, *14*(6), e0218929.

Na, J., Kim, Y., Song, J., Shim, T., Cho, K., Jung, J. (2021). Evaluation of the combined effect of elevated temperature and cadmium toxicity on *Daphnia magna* using a simplified DEBtox model. *Environmental Pollution*, *291*, 118250.

Nagai, T., De Schamphelaere, K. A. (2016). The effect of binary mixtures of zinc, copper, cadmium, and nickel on the growth of the freshwater diatom Navicula pelliculosa and

comparison with mixture toxicity model predictions. *Environmental toxicology and chemistry*, *35*(11), 2765-2773.

Naka, K. S., dos Santos Mendes, L. D. C., de Queiroz, T. K. L., Costa, B. N. S., de Jesus, I. M., de Magalhães Câmara, V., de Oliveira Lima, M. (2020). A comparative study of cadmium levels in blood from exposed populations in an industrial area of the Amazon, Brazil. *Science of the total environment*, *698*, 134309.

Negilski, D. S., Ahsanullah, M., Mobley, M. C. (1981). Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis*. II. Effects of paired and triad combinations of metals. *Marine Biology*, *64*(3), 305-309.

Odate, S., Pawlik, J. R. (2007). The role of vanadium in the chemical defense of the solitary tunicate, Phallusia nigra. *Journal of chemical ecology*, *33*, 643-654.

Odobašić, A., Šestan, I., Begić, S. (2019). Biosensors for determination of heavy metals in waters. *Biosensors for environmental monitoring*.

P. H. Raven et al. The distribution of biodiversity richness in the tropics. (2020). Sci. Adv. 6, eabc6228

Perić, L., Perusco, V. S., Nerlović, V. (2020). Differential response of biomarkers in the native European flat oyster Ostrea edulis and the non-indigenous Pacific oyster Crassostrea gigas co-exposed to cadmium and copper. *Journal of Experimental Marine Biology and Ecology*, *523*, 151271.

Pfeiffer, W. A., & de Lacerda, L. D. (1988). Mercury inputs into the Amazon region, Brazil. *Environmental Technology*, *9*(4), 325-330.

Qian, H., Li, J., Pan, X., Sun, L., Lu, T., Ran, H., Fu, Z. (2011). Combined effect of copper and cadmium on heavy metal ion bioaccumulation and antioxidant enzymes induction in *Chlorella vulgaris*. *Bulletin of environmental contamination and toxicology*, *87*, 512-516.

Ramesh, K., Elango, L. (2014). Impact of groundwater quality from industrial East Coastal Town, Southern India. *Intern J Eng Res Appli*, *4*(1), 346-354.

Ramírez-Pérez, T., Sarma, S. S. S., Nandini, S. (2004). Effects of mercury on the life table demography of the rotifer *Brachionus calyciflorus* Pallas (Rotifera). *Ecotoxicology*, *13*, 535-544.

Ren, X., Wang, X., Liu, P., Li, J. (2019). Bioaccumulation and physiological responses in juvenile *Marsupenaeus* japonicus exposed to cadmium. *Aquatic Toxicology*, *214*, 105255.

Rice, E. W., Bridgewater, L., American Public Health Association (Eds.). (2012). *Standard methods for the examination of water and wastewater* (Vol. 10). Washington, DC: American public health association.

Rico, A., de Oliveira, R., de Souza Nunes, G. S., Rizzi, C., Villa, S., Vizioli, B. D. C., Waichman, A. V. (2022). Ecological risk assessment of pesticides in urban streams of the Brazilian Amazon. *Chemosphere*, *291*, 132821.

Rizzi, C., Villa, S., Waichman, A. V., de Souza Nunes, G. S., de Oliveira, R., Vighi, M., & Rico, A. (2023). Occurrence, sources, and ecological risks of polycyclic aromatic hydrocarbons (PAHs) in the Amazon river. *Chemosphere*, 139285.

Rocha, O., Neto, A. J. G., dos Santos Lima, J. C., Freitas, E. C., Miguel, M., da Silva Mansano, A., Daam, M. A. (2018). Sensitivities of three tropical indigenous freshwater invertebrates to single and mixture exposures of diuron and carbofuran and their commercial formulations. *Ecotoxicology*, *27*, 834-844.

Santana, G. P., & Barroncas, P. D. S. R. (2007). Estudo de metais pesados (Co, Cu, Fe, Cr, Ni, Mn, Pb e Zn) na Bacia do Tarumã-Açu Manaus (AM). *Acta Amazonica*, *37*, 111-118.

Sevilla, J. B., Nakajima, F., & Yamamoto, K. (2013). Effect of food and light on the sensitivity of copper and zinc to freshwater benthic ostracod *Heterocypris incongruens*. *Journal of Water and Environment Technology*, *11*(3), 249-261.

Sevilla, J. B., Nakajima, F., Kasuga, I. (2014). Comparison of aquatic and dietary exposure of heavy metals Cd, Cu, and Zn to benthic ostracod *Heterocypris incongruens*. *Environmental toxicology and chemistry*, *33*(7), 1624-1630.

Shakir, S. K., Azizullah, A., Murad, W., Daud, M. K., Nabeela, F., Rahman, H., Häder, D. P. (2017). Toxic metal pollution in Pakistan and its possible risks to public health. *Reviews of Environmental Contamination and Toxicology Volume 242*, 1-60.

Sharma, A., Grewal, A. S., Sharma, D., Srivastav, A. L. (2023). Heavy metal contamination in water: consequences on human health and environment. In *Metals in Water* (pp. 39-52). Elsevier.

Shuhaimi-Othman, M., Pascoe, D. (2001). Acute toxicity of copper, zinc and cadmium to the freshwater amphipod *Hyalella azteca*. *Malaysian Applied Biology*, *30*(1/2), 1-8.

Shuhaimi-Othman, M., Yakub, N., Ramle, N. A., Abas, A. (2011). Toxicity of metals to a freshwater ostracod: *Stenocypris major*. *Journal of Toxicology, 2011*.

Silva, M. H. L., de Castro, A. C. L., da Silva, I. S., Cabral, P. F. P., de Jesus Azevedo, J. W., Soares, L. S., Nunes, J. L. S., (2023). Determination of metals in estuarine fishes in a metropolitan region of the coastal zone of the Brazilian Amazon. *Marine Pollution Bulletin*, *186*, 114477.

Taslima, K., Al-Emran, M., Rahman, M. S., Hasan, J., Ferdous, Z., Rohani, M. F., Shahjahan, M. (2022). Impacts of heavy metals on early development, growth and reproduction of fish–A review. *Toxicology Reports*, *9*, 858-868.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology: volume 3: environmental toxicology*, 133-164.

Thorley, J., Schwarz C. (2018). ssdtools: An R package to fit Species Sensitivity Distributions. *Journal of Open Source Software*, 3 (31), 1082.

USEPA - US Environmental Protection Agency. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Washington, DC. http://www.epa.gov/pesticides/bluebook/chapter1.html

Van Vlaardingen, P., Traas, T., Wintersen, A., Aldenberg, T., (2004). A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. *National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands*.

Vatandoost, M., Naghipour, D., Omidi, S., Ashrafi, S. D., (2018). Survey and mapping of heavy metals in groundwater resources around the region of the Anzali International Wetland; a dataset. *Data in brief*, *18*, 463-469.

Viana, L. F., Kummrow, F., Cardoso, C. A. L., de Lima, N. A., Solórzano, J. C. J., do A. Crispim, B., Florentino, A. C. (2021). High concentrations of metals in the waters from Araguari River lower section (Amazon biome): Relationship with land use and cover, ecotoxicological effects and risks to aquatic biota. *Chemosphere*, 285, 131451.

Wang, C., Kuang, X., Shan, J., Zhang, Q., Zhou, Z., Tong, Y., Yiguang, Z. (2022). Recent ostracods as ecological indicators and its applications: An example from the southern Tibetan Plateau (2022). *Ecological Indicators* 143(6):109326

Watson, G. J., Pini, J. M., Richir, J. (2018). Chronic exposure to copper and zinc induces DNA damage in the polychaete *Alitta virens* and the implications for future toxicity of coastal sites. *Environmental Pollution*, *243*, 1498-1508.

Wu, X., Cobbina, S.J., Mao, G., Xu, H., Zhang, Z., Yang, L., (2016). A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. *Environ. Sci. Pollut. Res.* 23, 8244–8259.

Xu, X., Shi, L., Wang, M. (2016). Comparative quantitative proteomics unveils putative mechanisms involved into mercury toxicity and tolerance in *Tigriopus japonicus* under multigenerational exposure scenario. *Environmental Pollution*, *218*, 1287-1297.

Yan, F., Li, N., Yang, Z., Qian, B., 2022. Ecological Risk Evaluation of Baihetan Dam Based on Fuzzy Hazard Quotient Model. *Water*, *14* (17), 2694.

Yoo, J. W., Jeon, M., Lee, K. W., Jung, J. H., Jeong, C. B., Lee, Y. M. (2022). The single and combined effects of mercury and polystyrene plastic beads on antioxidant-related systems in the brackish water flea: Toxicological interaction depending on mercury species and plastic bead size. Aquatic Toxicology, 252, 106325.

Yuan, D., Huang, L., Zeng, L., Liu, S., He, Z., Zhao, M., Qin, C. (2017). Acute toxicity of mercury chloride (HgCl2) and cadmium chloride (CdCl2) on the behavior of freshwater fish, *Percocypris pingi*. *International Journal of Aquaculture and Fishery Sciences*, 3(3), 066-070.

Zeeshan, M., Murugadas, A., Ghaskadbi, S., Rajendran, R. B., Akbarsha, M. A. (2016). ROS dependent copper toxicity in Hydra-biochemical and molecular study. Comparative Biochemistry and Physiology Part C: *Toxicology & Pharmacology*, 185, 1-12.

Zhang, J., Yang, W., Li, Z., Huang, F., & Zhang, K. (2023) Multigenerational exposure of cadmium trans-generationally impairs locomotive and chemotactic behaviors in Caenorhabditis elegans. *Chemosphere*, 325, 138432.

Chapter 4 - Integrated Response of Biomarkers of stress and oxidative damage in sublethal exposures to different metals for the ostracod *Strandesia rondoniensis*

Integrated Response of Biomarkers of stress and oxidative damage in sublethal exposures to different metals for the ostracod *Strandesia rondoniensis*

Abstract

Metals are carried in different forms to aquatic ecosystems due to human activities, which have caused serious problems for aquatic biota. Among the many adverse effects, oxidative stress has been widely used as an indicator of environmental pollution. From this perspective, this study evaluated different biomarkers of stress and oxidative damage for metallic salts of copper (Cu), zinc (Zn), cadmium (Cd), and mercury (Hg), using as a test organism a species of ostracod collected in the Amazon, *Strandesia rondoniensis*. The sublethal experiment (96h) was conducted by exposing *S. rondoniensis* to the following concentrations (mg L^{-1}): $CuSO_4$ (0; 0.001; 0.01; 0.1), $ZnCl_2$ (0; 0.01; 0.1; 1), $CdCl_2$ (0; 0.001; 0.01), $HgCl_2$ (0; 0.001; 0.01; 0.05). Biomarker analyzes revealed that the activity of the superoxide dismutases, glutathione, and metallothionein were increased in organisms exposed to the metals Zn, Cd, and Hg. It is possible to associate that these metals led to the production of reactive oxygen species (ROS) in ostracod. In the other way, any of the biomarkers analyzed were altered after exposure to Cu. The increase in lipid peroxidation provoked by Zn, Cd and Hg shows damage to the plasma membrane, revealing that the antioxidant defense systems were not efficient in protecting cells against the ROS, with provoked DNA damages. The data presented in this study are unprecedented, as it is the first time that an ostracod species native to the Amazon has been used as a test organism in ecotoxicology, and the use of biomarkers for this group is even scarcer. The response of *S. rondoniensis* to the different biomarkers is satisfactory and reinforces the different findings available in the literature. In this way, we reinforce the importance of reviewing the limits established by current environmental legislation from Brazil and the use of neotropical species as test organisms. Furthermore, it is evidenced that the continuous monitoring of ecosystems using biomarkers is an efficient technique for responding to adverse effects caused due to chronic exposure to metals.

Keywords: Amazon, cadmium, copper, native species, mercury, zinc.

1. Introduction

The Amazon is one of the biomes with the greatest biodiversity in the world (Rico et al., 2022). However, in recent decades, population density in small and large urban centers (Souza, 2000) has generated the continuous entry of domestic and industrial effluents into aquatic ecosystems (Gomes et al., 2023), mainly due to the low basic sanitation coverage in the Northern region of Brazil (Brazil, 2023). In addition, illegal mining activities (Moulatlet et al., 2023) have caused an excessive entry of metals into the aquatic ecosystems of this region.

When metals enter the aquatic ecosystem, they can cause serious damage to biota (Kahlon et al., 2018; Jeong et al., 2023) since, even in sublethal concentrations (Ali et al., 2019), they are capable of interacting with biota, which can bioaccumulate then (Kuehr et al., 2021), cause adverse effects on growth and reproduction (Liang et al., 2022; Rebolledo et al., 2022), immunotoxicity (Ray et al., 2015), or even lead to oxidative stress, caused by the production of reactive oxygen species (ROS) (Watson, Pini, Richir, 2018; Jeong et al., 2023), which cause damage to different macromolecules such as lipids, proteins, and DNA (Jeong et al. al., 2023).

Cells, in turn, have an efficient enzymatic defense system (*e.g.* superoxide dismutase – SOD; catalase – CAT; glutathione peroxidase – GPx; among others) and non-enzymatic (e.g. glutathione – GSH; metallothionein – MT) to cope with these metabolites. Under normal physiological conditions, the adverse effects of ROS are neutralized by the antioxidant cellular defense system (Dandapat, Chainy, Rao 2000), being mainly responsible for the inhibition or reduction of their damage in cells (Cerutti , 1994). On the other hand, if this system fails, lipid peroxidation (LPO) represents one of the main mechanisms of oxidative injury used as a biomarker of effects on living organisms (Di Giulio and Meyer, 2008).

From this perspective, the use of different biomarkers in environmental monitoring represents an advance in understanding the effects caused by metal pollution (Barata et al., 2005). Although biomarkers are established as an effective technique for verifying adverse effects at different trophic levels (Lee et al., 2021; Kim et al., 2022), studies with aquatic invertebrates are still scarce, especially with ostracods. In this way, a single study that identified genes linked to antioxidant activities for the ostracod *Heterocypris incongruens* was identified (Hiki et al., 2019). Therefore, it is important to assess defense and oxidative damage systems in different species of invertebrates to

establish appropriate methods to validate in advance the effects of oxidative stress on aquatic biota (Barata et al., 2005).

Based on the above, this study assessed the enzymatic and non-enzymatic biomarkers, oxidative stress, and DNA damage through the markers: SOD, GSH, MT, LPO, and DNA-StandBreak for the species *S. rondoniensis*, after sublethal exposure to environmentally relevant concentrations of metallic salts copper sulfate, zinc-, cadmium- and mercury-chloride.

2. **Materials and methods**

2.1 Test organism sampling and maintenance

The organisms were collected in August 2018 in the Porto Velho Natural Park, Rondônia State (8º 41’ 13,26” S; 63º 52’ 10,06” O), with the SISBio authorization (Nº: 67974-1) and taken to the Laboratory of Limnology and Aquatic Ecotoxicology at the Federal University of São Carlos – UFSCar. After collection, the organisms were acclimatized in the laboratory in 4L plastic. Cultures were maintained under controlled conditions, with a 12h light / 12h dark light regime and temperature of 25 °C ± 1 °C. The detailed taxonomic description, as well as its life cycle description, were presented by Gomes et al. (2024).

In the laboratory, the cultures were maintained following adaptations from the protocol established for the tropical cladoceran species *Ceriodaphnia silvestrii* (ABNT, 2022, Nº 13373), using reconstituted water with the following parameters: pH 7-7.6, hardness 40-48 mg L^{-1} of $CaCO_3$, conductivity of 160 µS cm^{-1}. The diet was based on 20 mL of Tetramin® fish food (5g L^{-1}) and 20 mL of *Raphidocelis subcapitata* suspension (≈ 10^8 cells L^{-1}).

2.2 Preparation and Quantification of Stock Solutions

To evaluate the chronic effects of metals, the following salts were used: Copper Sulfate ($CuSO_4$, CAS No: 7758-98-8, Dinâmico), Cadmium Chloride ($CdCl_2$; Nº CAS: 35658-65-2; Carlo Erba Reagentes), Zinc Chloride ($ZnCl_2$; Nº CAS: 7646-85-7; Merck), and Mercury Chloride ($HgCl_2$; CAS: 7487-94-7; Merck). Stock solutions of each metal salt were prepared in distilled water at the concentration of 10 mg L^{-1}. The nominal concentrations of the tested substances were obtained by diluting the stock solution in reconstituted water. To confirm the concentrations of $CuSO_4$, $CdCl_2$, and $ZnCl_2$ an aliquot

from the stock solutions was taken, acidified (HNO_3 analytical grade) until pH < 2.0, and used to quantify real concentrations. These analyses were made in Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES Optima 8300/Perkin Elmer). Quantification followed recommendations from the 23rd edition of Standard Methods (SMWW, 3111 B). The quantification of $HgCl_2$ was carried out using the DMA-80 EVO Milestone mercury analyzer (USEPA, 7473).

2.3 Exposure of test organisms to metals

Three concentrations of each metallic salt were prepared, set as the no-observable effect concentration (NOEC) from the acute toxicity values presented by Gomes et al. (2024). Besides, in the present study, only environmentally relevant concentrations were tested. Therefore, the following concentrations were used: $CuSO_4$ (0; 0.001; 0.01; 0.1 mg L^{-1}), $ZnCl_2$ (0; 0.01; 0.1; 1 mg L^{-1}), $CdCl_2$ (0; 0.001; 0.01 mg L^{-1}) and $HgCl_2$ (0; 0.001; 0.01; 0.05 mg L^{-1}). The exposure was conducted in 250 mL non-toxic plastic containers, containing 100 mL of test solution. Only one experiment was carried out for each metal, with five replicates per concentration, containing 150 adult individuals each. The experiment lasted 96 hours and was maintained under controlled temperature (25 ± 1°C) in the dark. Food (2 mL of fish food solution) was provided at the beginning and after 48 hours. The water parameters pH, electrical conductivity (EC), temperature (T), and dissolved oxygen (DO) were measured at the beginning and end of the experiment.

2.4 Biomarker analysis

After 96 h of exposure, the biomass (0.001 mg) of the pool of all individuals from each replicate (n = 75) was determined and samples were immediately stored at -80 ºC for molecular and biochemical analyses. Then, the samples were homogenized by ultrasound (Eco-Sonics, QR500, Brasil) in a phosphate buffer 0.1 M (pH 7.6) and centrifuged at 10.000 × g for 10 min at 4°C. A 1:5 ratio of buffer to sample mass was used.

The protein concentration of the samples (pt, mg mL^{-1}) was quantified based on the Bradford method (1976) using bovine serum albumin as standard, and readings were taken at 595 nm. The protein concentration for each sample is presented in Table S3 and was used to standardize the enzyme activity.

The determination of metallothionein (MT) concentration was carried out using buffer Tris-HCl 20 mM with sucrose 0.5 mM and B-mercaptoetanol (Viarengo et al., 1997). The enzymatic activity of superoxide dismutase (SOD, U mg pt^{-1} min^{-1}) was quantified based on the inhibition of the reduction rate of cytochrome C by superoxide radicals at 550 nm, according to the method described by McCord e Fridovich (1969). While the concentration of glutathione (GSH, nmol GSH mg pt^{-1} min^{-1}) was quantified by the reaction of 2.3-naftalenodicarboxaldeído (NDA) with GSH for the formation of a fluorescent compound, which was measured based on excitation/emission wavelengths of 472/528 nm (White et al., 2003).

Lipid peroxidation (LPO, nmol CHP mg pt^{-1}) was quantified using the xylenol orange ferrous oxidation method (FOX), based on the measurement of absorbance at 560 nm according to the protocol presented by Jiang et al. (1991, 1992).

Finally, to assess DNA damage related to oxidative stress, the alkaline precipitation protocol (Olive, 1988) was used to quantify breaks in the DNA chain (µg DNA/total proteína). Analyzes were performed with a microplate reader SpectraMax M5 (Multi-Mode, Molecular Devices, EUA).

2.5 Statistical analysis

Significant differences for each of the biomarkers in the different treatments about the control were evaluated using the Generalized Linear Models (GLM) with the Gaussian family (identity link-function). The analyses were carried out using the Jamovi software, Version 2.4 (Galluci, 2019), with a confidence level of 95%, considering statistical differences for a p-value < 0.05. Principal component analysis (PCA) was performed using the 'FactoMineR' and 'factoextra' packages in the R Core Team software (R core team, 2020) to generate two-dimensional biplots. The plots were generated by the "ggplot2" package.

3. **Results**

3.1 Survival

Exposure to any of the tested concentrations of $CuSO_4$, $ZnCl_2$, and $HgCl_2$ provoked effects in the *S. rondoniensis* survival after 96 h ($p > 0.05$). On the other way, $CdCl_2$ reduced the survival of the species at 0.1 mg L^{-1} compared with control. For this

reason, biomarkers were not studied regarding this treatment, once the objective was to assess the sublethal responses.

3.2 Water parameters of test solutions

The water parameters at the beginning and end of the experiments remained stable, with the pH varying between 7.4-7.8, the electrical conductivity between 133.4-216 μS cm^{-1}, the dissolved oxygen between 4.3-6.9 mg L^{-1}, the temperature between 24.5-25.3 °C and the hardness between 40-48 mg $CaCO_3$ L^{-1} (Table S2). The concentrations of metals used in this study did not vary by more than 10% from the nominal ones (Table S1). Therefore, nominal values will be presented throughout the description of the results, as recommended by the ISO standard (2000).

3.3 Antioxidant defense biomarkers

The activity of the antioxidant enzyme SOD was higher than in the control group ($p < 0.05$) in the concentration of 0.01 mg L^{-1} of $CdCl_2$, and 0.05 mg L^{-1} of $HgCl_2$. While for Cu and Zn salts, no effects were observed (Figure 1, $p > 0.05$). On the other hand, GSH content increased in *S. rondoniensis* individuals exposed to $ZnCl_2$, $CdCl_2$ e $HgCl_2$ ($p<0.05$, Figure 2), in concentrations 1; 0.01; 0.01 respectively. For $HgCl_2$, only two treatments were analyzed since the amount of sample from 0.01 and 0.05 mg L^{-1} available for this analysis was insufficient.

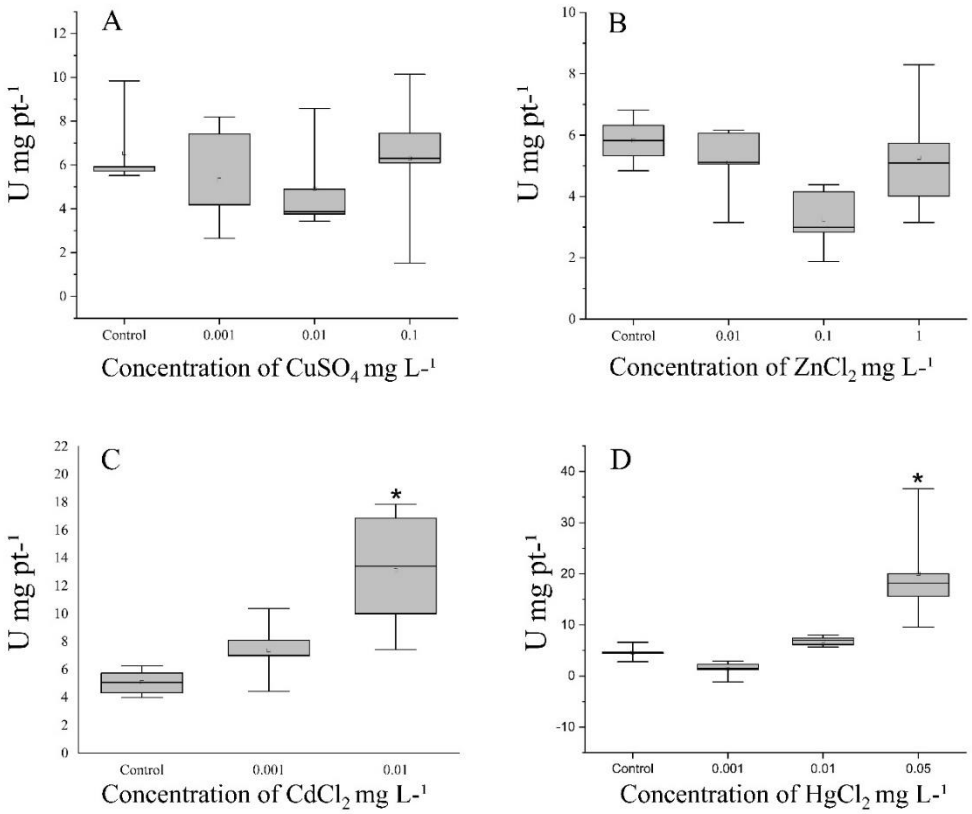


Figure 1 – Responses of the enzymatic activity superoxide dismutase (SOD – U mg pt-1) for the species *S. rondoniensis*, for the different metals analyzed (A – $CuSO_4$; B – $ZnCl_2$; C – $CdCl_2$; D – $HgCl_2$) in 96 h exposure. Asterisks (*) indicate statistically significant differences from the control ($p<0.05$).

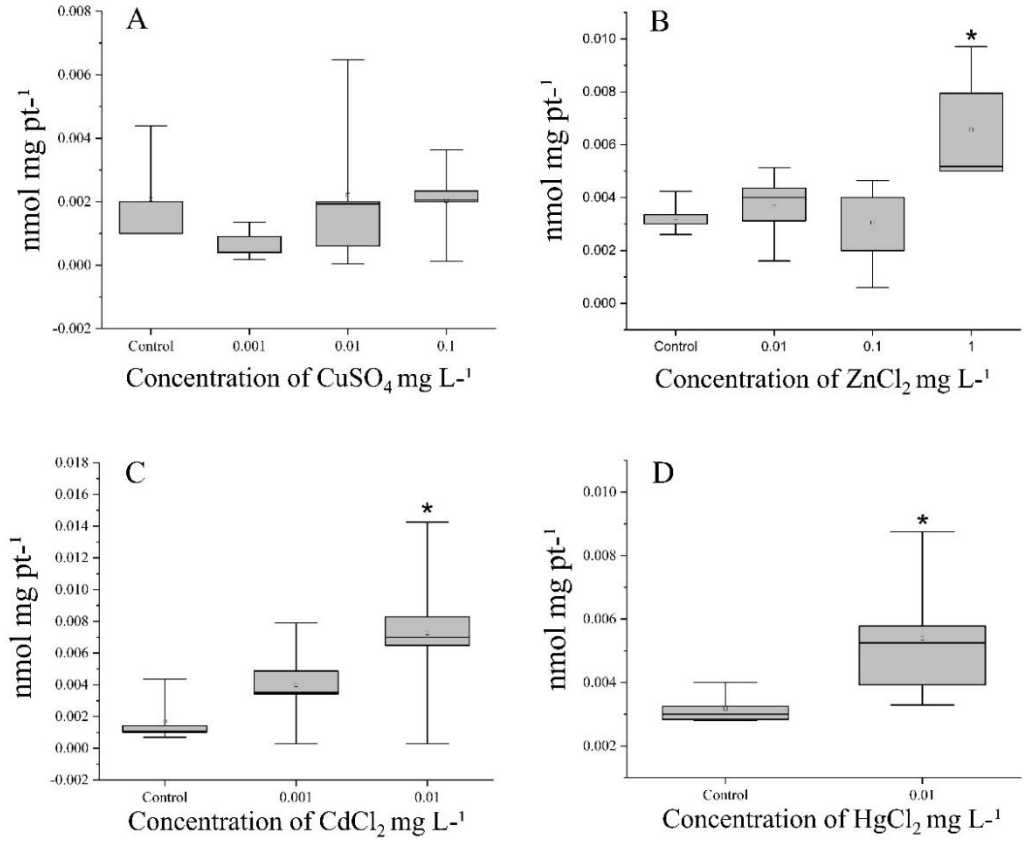


Figure 2 – Concentration of the non-enzymatic antioxidant glutathione (GSH – nmol mg pt^{-1}) for the species . *rondoniensis*, for the different metals analyzed (A – $CuSO_4$; B – $ZnCl_2$; C – $CdCl_2$; D – $HgCl_2$) in 96 h exposure. Asterisks (*) indicate statistically significant differences from the control (p<0.05).

MT levels were significantly higher for the metals Zn, Cd, and Hg, at concentrations 0.01 and 1, 0.001, and 0.05 mg L^{-1}, respectively, regarding control (p<0.05, Figure 3).

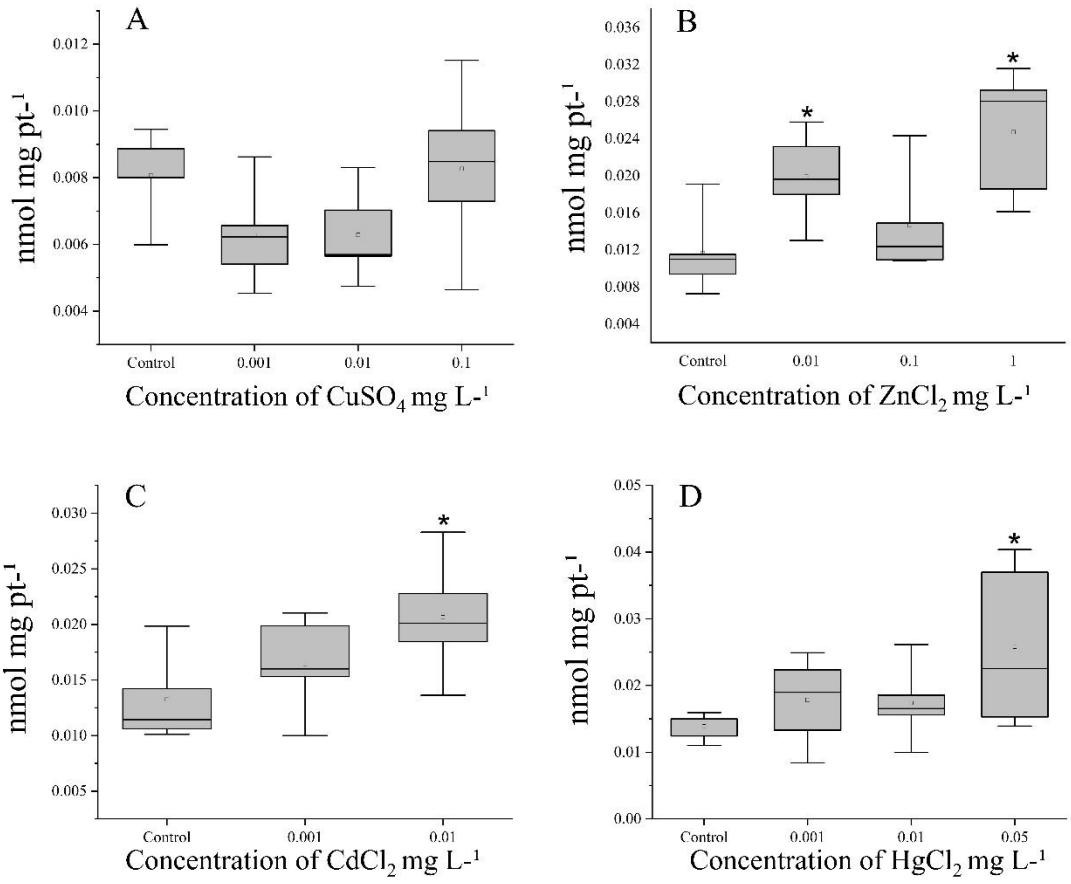


Figure 3 – Quantification of metallothionein (MT) for the species *S. rondoniensis*, for the different metals analyzed (A – $CuSO_4$; B – $ZnCl_2$; C – $CdCl_2$; D – $HgCl_2$) in 96 h exposure. Asterisks (*) indicate statistically significant differences from the control (p<0.05).

3.4 Biomarkers of stress and oxidative damage

The quantification of lipid peroxidation (LPO) showed that there was a significant increase (p<0.05) in the levels after exposure to metal salts $ZnCl_2$ (1 mg L^{-1}), $CdCl_2$ (0.01 mg L^{-1}), and $HgCl_2$ (0.01 and 0.05 mg L^{-1}) (Figure 4).

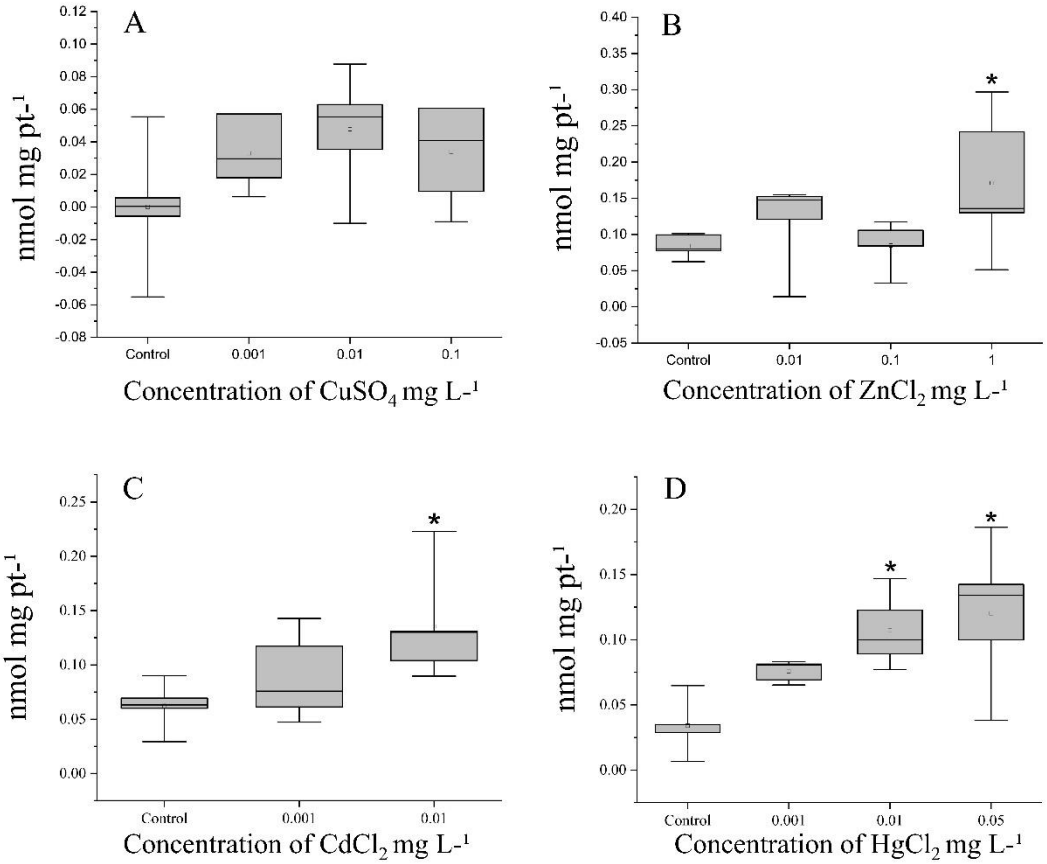


Figure 4 – Lipid peroxidation (LPO) responses for the species *S. rondoniensis*, for the different metals analyzed (A – $CuSO_4$; B – $ZnCl_2$; C – $CdCl_2$; D – $HgCl_2$). Asterisks (*) indicate statistically significant differences from the control ($p<0.05$).

DNA-StandBreak analysis showed that metals Zn, Cd, and Hg provoked increases ($p<0.05$) compared with control in the concentrations of 1 mg L^{-1} of $ZnCl_2$, 0.001 and 0.01 mg L^{-1} of $CdCl_2$, and 0.05 mg L^{-1} of $HgCl_2$ (Figure 5).

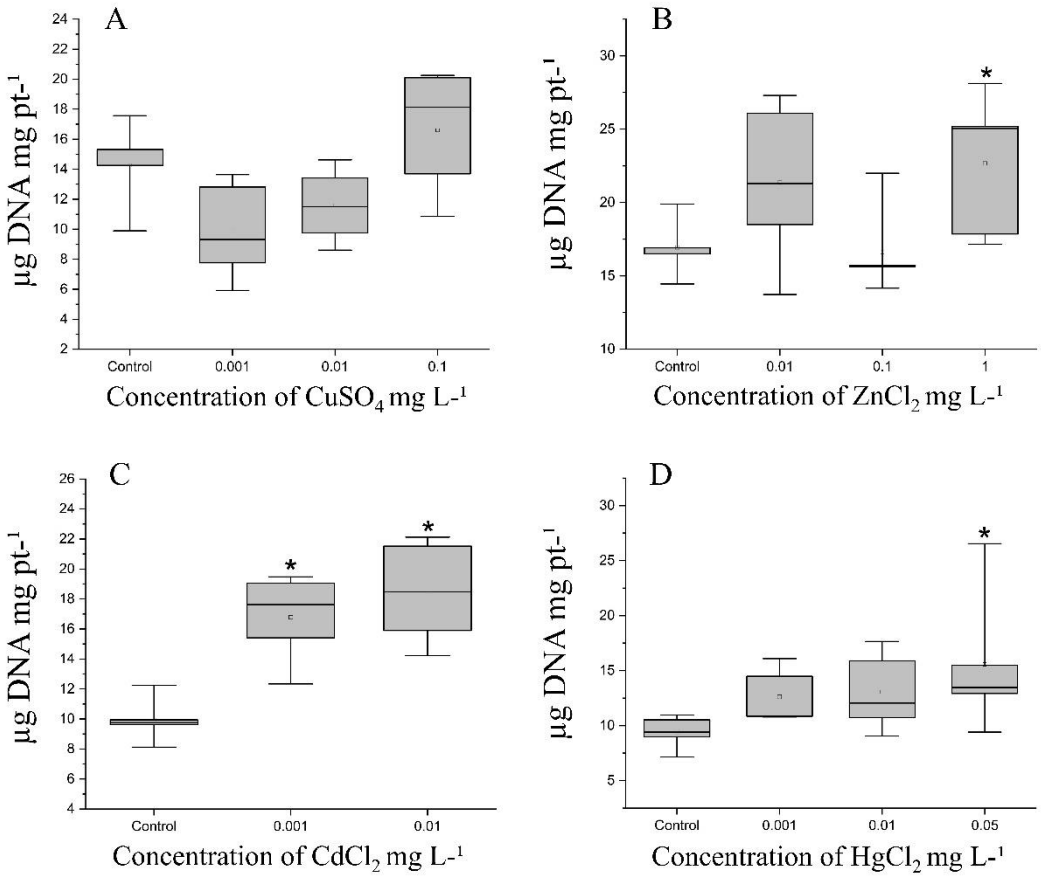


Figure 5 – DNA damage responses for the species *S. rondoniensis*, for the different metals analyzed (A – $CuSO_4$; B – $ZnCl_2$; C – $CdCl_2$; D – $HgCl_2$) (* = p<0.05).

In the PCA analysis, Dim1 accounted for 32.3% of the data variability, while Dim2 19.4% (Figure 6). The analysis shows that the metals Hg and Cd had a clear relationship with the markers SOD, MT, and LPO, while Zn had a greater relationship with GSH and DNA-StandBreak. Cu, in turn, showed a low relationship with all the variables analyzed. It is also possible to observe that control (C1) and the lower concentration of each metal (C2) had little relationship with the markers, while the higher concentrations (C3 and C4) strongly influenced all biomarkers.

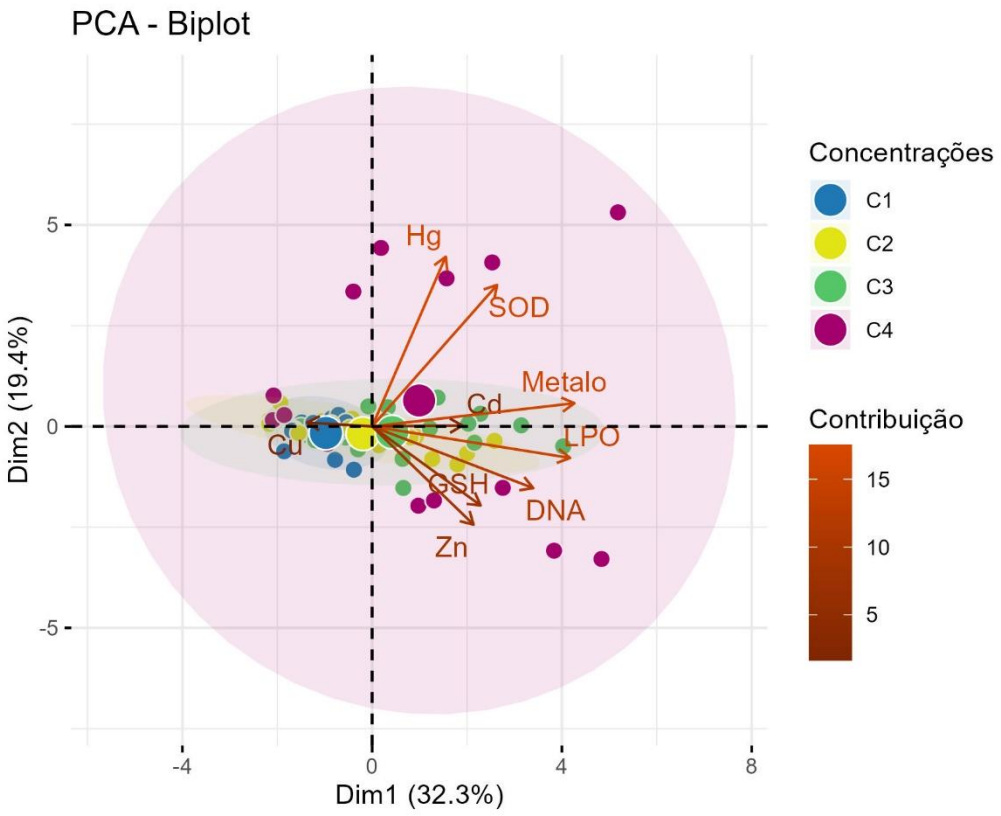


Figure 6 - PCA analysis with the concentrations of metals in water samples from the Amazon Basin. The DIM1 accounted for 32.3% of all variation, and the DIM2 for 19.4%. In which, (C1 = 0 for all metals; C2 = 0.001, 0.01, 0.001, 0.001; C3 = 0.01, 0.1, 0.01; C4 = 0.1, 1, 0.05 for metals $CuSO_4$, $ZnCl_2$, $CdCl_2$, $HgCl_2$, respectively), SOD – superoxide dismutase; Metalo – Metallothionein; DNA-StandBreak - DNA; Glutatione – GSH; lipid peroxidation – LPO.

4. Discussion

4.1 Defense mechanisms and antioxidant enzymes

Metal pollution, whether in water or sediment, has aroused concern in the scientific community for decades. Since it is already established that metals cause the production of ROS, mainly the hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2), through the uncoupling of the Haber-Weiss and Fenton reactions, from the interaction of metals in their ionic form with molecular oxygen (Leonard et al., 2004), leading to oxidative stress (Ren et al., 2019).

Exposed organisms present antioxidant defense mechanisms to protect cells against ROS attack. This mechanism includes enzymes such as superoxide dismutase (SOD) (Livingstone, 2003), which convert superoxide radicals (O_2^-) in H_2O and O_2 (Li et al., 2015). SOD activity represents one of the main antioxidant enzyme systems in aquatic invertebrates (Barata et al., 2005), being considered the first line of defense against the toxicity of xenobiotics in organisms (Pandey et al., 2003), while GSH serves as an important substrate for the action of antioxidant and detoxification enzymes such as GST, GPx and GR (Farombi, Adelowo, Ajimoko, 2007; Frias-Espericueta et al., 2022).

The metals Cd and Hg increased SOD levels, while Zn, Cd, and Hg caused an increase in GSH in exposed individuals. The increase in SOD is related to the increase in ROS, caused by metal toxicity (John et al., 2001). On the other hand, studies conducted by Kang (1997) demonstrated that cellular GSH levels can increase after prolonged exposure to metals. This suggests that the increase in GSH production is a compensatory response to the high body load of metals, serving as a substrate for the action of antioxidant enzymes and helping to detoxify the cells (Wilczek et al., 2004), since GSH plays a fundamental role in non-enzymatic antioxidant defense (Mahboob, 2013).

Data presented by Ren et al. (2019) indicated that concentrations of 5 to 50 µg L^{-1} of Cd caused an increase in GSH content and SOD activity for the crustacean *Marsupenaeus japonicus.* Chen et al. (2014), reported that Hg led to increased expression of genes linked to SOD in sublethal exposures to the mollusk *Venerupis philippinarum*. Therefore, the increase in SOD levels coupled with the increase in GSH, mainly for Zn, Cd, and Hg, shows that *S. rondoniensis* had an increase in ROS production, stimulating the antioxidant defense systems.

Metallothioneins (MT) are non-enzymatic proteins, with the ability to bind metals through thiol groups on cysteine residues, protecting organims against metal toxicity (Sevcikova et al., 2011). They perform special functions in metal detoxification and have a fundamental role in the metabolism and homeostasis of essential metals (Kelly et al., 1998). MTs have been found in a wide variety of invertebrates, including mollusks, annelid worms, and crustaceans (Amiard et al., 2006).

In this study, the metals Zn, Cd, and Hg increased MT concentrations. This increase in MT has already been reported for other organisms exposed to metals, as demonstrated by Gunderson et al. (2021), who found that low concentrations of Zn caused an increase in MT concentrations in crayfish *Pacifastacus leniusculus*. Verlecar et al. (2008), observed a significant increase in the level of MT in the digestive gland of the

mussel *Perna viridis* exposed to 45 μg L^{-1} of Hg. Furthermore, exposure to 20 μg L^{-1} of Hg induced a significant increase in MT in the gills of the oyster *Crassostrea gigas* and the mussels *Mytilus edulis* in 21 days of exposure (Géret et al., 2002). On the other hand, Cd also induced MT expression after 4 days of exposure in the gills of the oyster *Crassostrea virginica* exposed to 50 μg Cd L^{-1} (Roesijadi e Klerks, 1989). Therefore, the data presented in this study reveal that *S. rondoniensis* exposed to different metals also increased their MT levels in response to high concentrations, thus using this mechanism to detoxify the metals.

4.2 Stress and oxidative damages

Lipid peroxidation (LPO) occurs when excessive amounts of ROS are cumulated in cells, interacting with membrane lipids and thus initiating excessive production of lipid peroxides, disrupting normal cell function (Regoli and Giuliani, 2014). It is one of the main biomarkers of oxidative stress (Di Giulio and Meyer, 2008), indicating loss of important cellular functions (Storey, 1996), such as cell membrane permeability disorders, changes in ionic flux, loss of selectivity in the exchange of nutrients and toxic substances into the cell, oxidation of lipoproteins and changes in DNA (Baber & Harris, 1994; Di Giulio and Meyer, 2008).

In general, organisms from polluted environments show an increase in LPO values (Ferreira et al., 2005). In the present study, the increase in LPO levels indicates oxidative stress induced by the metals Zn, Cd, and Hg. Contrary, Cu does not induce significant responses to any of the investigated biomarkers. The damage caused by lipid oxidation harms several physiological processes, as previously mentioned. The LPO response observed in this study is in line with the data presented by Oliveira et al. (2018), who found an increase in LPO when the bivalve *Corbicula fluminea* was exposed to mercury. Zn led to an increase in LPO in sublethal exposures with the mollusk *Theba pisana* (Radwan, Gendy, and Gad, 2010). In the same way, exposure to 0.9 μg L^{-1} of Cd increased the LPO in the gills of *Bathymodiolus azoricus* (Company et al., 2004).

DNA damage has been used as a biomarker to assess the genotoxicity of metals (Bolognesi e Cirillo, 2014), which was evidenced in the present study in organisms exposed to higher concentrations of Zn, Cd, and Hg. The response to damage to DNA strands is consistent with the other results presented, as the increase in SOD, GSH, and MT shows that the exposed organisms sought to reduce ROS within the cell generated by metal detoxification. However, the antioxidant and defense system failed to prevent

oxidative damage, as indicated by the significant increase in LPO levels, resulting in DNA damage. Evidence this process provoked by Cd has already been presented by Chandra and Bukhsh (2004), in which they found genotoxicity in tissues of the *Oreochromis mossambicus.* Furthermore, a study with the amphipod *Gammarus elvirae* evidenced significant DNA damage in hemocytes after 24 hours of exposure to 0.1 µg L^{-1} of Hg (Donato et al., 2016).

The responses verified in this study are alarming, as the effects observed for the different biomarkers are within the standard established by Brazilian law, which establishes acceptable limits for the release of effluents allowing concentrations of 0.2 mg L^{-1} for Cd; 1.0 mg L^{-1} for Cu; 5.0 mg L^{-1} for Zn; and 0.01 mg L^{-1} for Hg (Brazil, 2011). On the other hand, the law responsible for establishing the classification and environmental guidelines for waterbodies is more restrictive for many metals (Brasil, 2005). In studies conducted by Gomes et al. (2023), it was found that around 44% of all metals evaluated in water in the Amazon biome were above these limits established. A study published by Moulatlet et al. (2023) reported maximum concentrations of metals in water samples in the Amazon influenced by mining activities, with concentrations reaching 1 mg L^{-1} for Cu, 2 mg L^{-1} for Zn, 0.043 mg L^{-1} for Cd, and 0.1 mg L^{-1} for Hg.

The high concentrations mentioned above represent a risk not only for aquatic biota (Viana et al., 2021; Gomes et al., 2023) but also for populations that inhabit the Amazon biome (Gomes et al., 2020; Galarza et al., 2022). Our results show that a reformulation of the limits established by law is necessary, as there is already concrete scientific evidence of the serious situation of the presence of metals in Amazonian aquatic ecosystems (Gomes et al., 2023; Moulatlet et al., 2023). However, biomarker studies for an ostracod native to the Amazon do not currently exist, and this is the first to propose this approach as a possible model for the use of biomarkers in environmental monitoring, as the ostracod *S. rondoniensis* demonstrated satisfactory responses to different biomarkers of stress and oxidative damage that were evaluated.

5. **Conclusion**

The results obtained in this study highlight, for the first time, the response of an ostracod native to the Amazon to different biomarkers of antioxidant defense, oxidative stress, and cellular damage. Based on these results, we can state that: I – Cu was the only metal that did not cause changes in the antioxidant defense biomarkers of the test organism. This indicates that even at low concentrations, with environmental relevance, the metals salts of Zn, Cd, and Hg caused an increase in ROS in cells. II – The increase in LPO levels indicates that the antioxidant defense systems were not sufficient to prevent damage to plasma membranes. III – The sum of these factors led to DNA damage, which in the long term could result in adverse effects on organisms at the individual and population level if this damage is persistent and DNA repair mechanisms are not efficient. In this way, this study highlights the importance of using biomarkers in environmental monitoring. Since, the responses observed in this study are valuable and help in decision-making, serving as a basis for the reformulation of environmental laws and more protective public policies.

References

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of chemistry, 2019*.

Amiard, J. C., Amiard-Triquet, C., Barka, S., Pellerin, J., & Rainbow, P. S. (2006). Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. *Aquatic toxicology*, *76* (2), 160-202.

Barata, C., Lekumberri, I., Vila-Escalé, M., Prat, N., Porte, C., 2005. Trace metal concentration, antioxidant enzyme activities and susceptibility to oxidative stress in the tricoptera larvae Hydropsyche exocellata from the Llobregat River basin (NE Spain). Aquat. Toxicol. 74, 3–19.

BARBER, A., & HARRIS, S. (1994) Oxygen free radicals and oxidants: a review. Am. Pharm., v. 34, p. 26-35.

BARBER, A., & HARRIS, S. Oxygen free radicals and oxidants: a review.

Bolognesi, C., & Cirillo, S. (2014). Genotoxicity biomarkers in aquatic bioindicators. *Current* Zoology, 60(2), 273-284.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https:// doi. org/ 10. 1016/0003- 2697(76) 90527-3

Brasil - Conselho Nacional Do Meio Ambiente (CONAMA), (2005). Resolução no 357, de 17 de março de. Ministério do Meio Ambiente. n◦ 053, pp. 58–63.

Brasil - Conselho Nacional Do Meio Ambiente (CONAMA), (2011). Resoluç˜ao no 430, de 30 de maio de 2011. Ministério do Meio Ambiente. n◦ 053.

Brasil - Ministério da Integração e do Desenvolvimento Regional. (2023). *Esgotamento Sanitário.* Disponível *em:*. https://www.gov.br/mdr/pt-br/assuntos/saneamento/snis/painel/es.

Ceretti, P.A. (1994). Oxy-radicals and cancer. Lancet, v. 344, p. 862-863.

Chandra, P., Khuda-Bukhsh, A. R. (2004). Genotoxic effects of cadmium chloride and azadirachtin treated singly and in combination in fish. *Ecotoxicology and environmental safety*, *58*(2), 194-201.

Chen, X., Zhang, R., Li, C., Bao, Y. (2014). Mercury exposure modulates antioxidant enzymes in gill tissue and hemocytes of *Venerupis philippinarum*. *Invertebrate Survival Journal*, *11*(1), 298-308.

Company, R., Serafim, A., Bebianno, M. J., Cosson, R., Shillito, B., Fiala-Medioni, A. (2004). Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel Bathymodiolus azoricus. *Marine environmental research*, *58*(2-5), 377-381.

Dandapat, J., Chainy, G.B.N., Rao, K.J. (2000). Dietary vitamin E modulates antioxidante defence system in giant freshwater prawn, *Macrobrachium rosenbergii*. Comp. Biochem. Physiol., 123c, p. 101-115.

Di Donato, G.; De Matthaeis, E.; Ronci, L.; Setini, A. (2016). Genotoxicity biomarkers in the amphipod *Gammarus elvirae* exposed in vivo to mercury and lead, and basal levels of DNA damage in two cell types. *Chemistry and Ecology, 32(9), 843–857.*

Di Giulio, R. T., Meyer, J. N. (2008). Reactive oxygen species and oxidative stress. *The toxicology of fishes*, 273-324.

Farombi, E. O., Adelowo, O. A., Ajimoko, Y. R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (*Clarias gariepinus*) from Nigeria Ogun River. *International journal of environmental research and public health*, *4*(2), 158-165.

Ferreira, M., Moradas-Ferreira, P., Reis-Henriques, M. A. (2005). Oxidative stress biomarkers in two resident species, mullet (*Mugil cephalus*) and flounder (*Platichthys flesus*), from a polluted site in River Douro Estuary, Portugal. *Aquatic toxicology*, *71*(1), 39-48.

Frías-Espericueta, M. G., Bautista-Covarrubias, J. C., Osuna-Martínez, C. C., Delgado-Alvarez, C., Bojórquez, C., Aguilar-Juárez, M., Páez-Osuna, F. (2022). Metals and oxidative stress in aquatic decapod crustaceans: A review with special reference to shrimp and crabs. *Aquatic Toxicology*, *242*, 106024.

Galarza, E., Moulatlet, G. M., Rico, A., Cabrera, M., Pinos-Velez, V., Pérez-González, A., Capparelli, M. V. (2023). Human health risk assessment of metals and metalloids in mining areas of the Northeast Andean foothills of the Ecuadorian Amazon. *Integrated Environmental Assessment and Management*, *19*(3), 706-716.

Gallucci, M. (2019). *GAMLj: General analyses for linear models*. [jamovi module]. Retrieved from https://gamlj.github.io/.

Géret F, Jouan A, Turpin V, Bebianno MJ, Cosson RP (2002). Influência da exposição ao metal na síntese de metalotioneína e peroxidação lipídica em dois moluscos bivalves: a ostra (*Crassostrea gigas*) e o mexilhão (*Mytilus edulis*). Recurso Vivo Aquat 15:61–66

Gomes, D. F., da Silva Pinto, T. J., Raymundo, L. B., da Fontoura Sperandei, V., Daam, M., Moreira, R. A., Rocha, O. (2023). Ecological risk assessment for metals in sediment and waters from the Brazilian Amazon region. *Chemosphere*, *345*, 140413.

Gomes, D. F., Moreira, R. A., Sanches, N. A. O., do Vale, C. A., Daam, M. A., Gorni, G. R., Bastos, W. R. (2020). Dynamics of (total and methyl) mercury in sediment, fish, and crocodiles in an Amazonian Lake and risk assessment of fish consumption to the local population. *Environmental monitoring and assessment*, *192*, 1-10.

Gomes, D. F. & Pereira J.S.; Pinto, R.L.; Moreira, R. Ap. M.; Rocha, O.; (2024). On a new species of ostracod from the Brazilian Amazon and its potential for experimental studies in laboratory culture. Submetido na "Limnologica".

Gomes, D. F. Brito, H.P.; Vale, J.G.; Pinto, R.L.; Moreira, R. Ap. M.; Rocha, O.; (2024). Toxicity of isolated and mixed metals to a native Amazonian ostracod and ecological risk assessment. Submetido na " Environmental Science and Pollution Research".

Gunderson, M. P., Boyd, H. M., Kelly, C. I., Lete, I. R., McLaughlin, Q. R. (2021). Modulation of endogenous antioxidants by zinc and copper in signal crayfish (Pacifastacus leniusculus). *Chemosphere*, *275*, 129982.

Hiki, K., Tsuchikawa, M., Nakajima, F., & Tobino, T. (2019). Identification of antioxidant genes in the ostracod *Heterocypris incongruens* through de novo transcriptome sequencing. *Journal of JSCE*, *7*(1), 133-142.

ISO - International Standard Organization (2000). Water quality – determination of long-term toxicity of substances to Daphnia magna Straus. ISO 10706, Paris

Jeong, H., Byeon, E., Kim, D. H., Maszczyk, P., Lee, J. S. (2023). Heavy metals and metalloid in aquatic invertebrates: A review of single/mixed forms, combination with other pollutants, and environmental factors. *Marine Pollution Bulletin*, *191*, 114959.

Jiang ZY, Hunt JV, Wolff SP (1992). Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 202:384–389.

Jiang ZY, Woollard AC, Wolff SP (1991). Lipid hydroperoxide measurement by oxidation of Fe2+in the presence of xylenol orange. Comparison with the TBA Assay and an Iodometric Method. J Lipids 26(10):853–856.

John, S., Kale, M., Rathore, N., Bhatnagar, D. (2001). Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *The Journal of nutritional biochemistry*, *12*(9), 500-504.

Kahlon, S. K., Sharma, G., Julka, J. M., Kumar, A., Sharma, S., Stadler, F. J. (2018). Impact of heavy metals and nanoparticles on aquatic biota. *Environmental chemistry letters*, *16*, 919-946.

Kang, Y.J., (1997). Alteration of antioxidant system. In: Masaro, E. (Ed.), Handbook of Human Toxicology. CRC Press, Boca Raton, Florida, USA, pp. 275–284.

Kelly, K. A., Havrilla, C. M., Brady, T. C., Abramo, K. H., Levin, E. D. (1998). Oxidative stress in toxicology: established mammalian and emerging piscine model systems. *Environmental health perspectives*, *106*(7), 375-384.

Kim, B. M., Kim, B., Nam, S. E., Eom, H. J., Lee, S., Kim, K., Rhee, J. S. (2022). Reductive transformation of hexavalent chromium in ice decreases chromium toxicity in aquatic animals. *Environmental Science & Technology*, *56*(6), 3503-3513.

Kuehr, S., Kosfeld, V., Schlechtriem, C. (2021). Bioaccumulation assessment of nanomaterials using freshwater invertebrate species. *Environmental Sciences Europe*, *33*, 1-36.

Lee, Y., Yoon, D. S., Lee, Y. H., Kwak, J. I., An, Y. J., Lee, J. S., Park, J. C. (2021). Combined exposure to microplastics and zinc produces sex-specific responses in the water flea Daphnia magna. *Journal of Hazardous Materials*, *420*, 126652.

Leonard, S.S., Harris, G.K., Shi, X. (2004). Metal-induced oxidative stress and signal transduction. Free Rad. Med., v. 37, p. 1921-1942.

Liang, Z., Chen, T., Yang, F., Li, S., Zhang, S., & Guo, H. (2022). Toxicity of chronic waterborne zinc exposure in the hepatopancreas of white shrimp Litopenaeus vannamei. *Chemosphere*, *309*, 136553.

Livingstone, D. R. (2003). Oxidative stress in aquatic organisms in relation to pollution and aquaculture.

Mahboob, S. (2013). Environmental pollution of heavy metals as a cause of oxidative stress in fish: a review. *Life Sci. J*, *10*, 336-347.

McCord JM, Fridovich I (1969). Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J Biol Chem 244:6049–6065. https:// doi. org/ 10. 1016/ S0021- 9258(18) 63504-5

Moulatlet, G. M., Yacelga, N., Rico, A., Mora, A., Hauser-Davis, R. A., Cabrera, M., Capparelli, M. V. (2023). A systematic review on metal contamination due to mining activities in the Amazon basin and associated environmental hazards. *Chemosphere*, 139700.

Olive PL (1988). DNA precipitation assay: a rapid and simple method for detecting DNA damage in mammalian cells. Environ Mol Mutagen 11(4):487–495.

Oliveira, P., Barboza, L. G. A., Branco, V., Figueiredo, N., Carvalho, C., Guilhermino, L. (2018). Effects of microplastics and mercury in the freshwater bivalve Corbicula fluminea (Müller, 1774): filtration rate, biochemical biomarkers and mercury bioconcentration. *Ecotoxicology and environmental safety*, *164*, 155-163.

Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B., Raisuddin, S. (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn.). *Science of the total environment*, *309*(1-3), 105-115.

R Core Team (2022). *R: A Language and environment for statistical computing*. (Version 4.1) [Computer software]. Retrieved from https://cran.r-project.org. (R packages retrieved from CRAN snapshot 2023-04-07).

R Core Team (2022). *R: A Language and environment for statistical computing*. (Version 4.1) [Computer software]. Retrieved from https://cran.r-project.org. (R packages retrieved from CRAN snapshot 2023-04-07).

Radwan, M. A., El-Gendy, K. S., Gad, A. F. (2010). Oxidative stress biomarkers in the digestive gland of Theba pisana exposed to heavy metals. *Archives of environmental contamination and toxicology*, *58*, 828-835.

Ray, S., Mukherjee, S., Bhunia, N. S., Bhunia, A. S., Ray, M. (2015). Immunotoxicological threats of pollutants in aquatic invertebrates. *Emerging Pollutants in the Environment—Current and Further Implications. InTech*, 149-167.

Rebolledo, U. A., Rico-Martínez, R., Fernández, R., Páez-Osuna, F. (2022). Synergistic effect of chloroquine and copper to the euryhaline rotifer Proales similis. *Ecotoxicology*, *31*(6), 1035-1043.

Regoli, F., Giuliani, M. E. (2014). Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. *Marine environmental research*, *93*, 106-117.

Ren, X., Wang, X., Liu, P., Li, J. (2019). Bioaccumulation and physiological responses in juvenile Marsupenaeus japonicus exposed to cadmium. *Aquatic Toxicology*, *214*, 105255.

Rico, A., de Oliveira, R., de Souza Nunes, G. S., Rizzi, C., Villa, S., Vizioli, B. D. C., Waichman, A. V. (2022). Ecological risk assessment of pesticides in urban streams of the Brazilian Amazon. *Chemosphere*, *291*, 132821.

Roesijadi, G., Klerks, P. L. (1989). Kinetic analysis of cadmium binding to metallothionein and other intracellular ligands in oyster gills. *Journal of Experimental Zoology*, *251*(1), 1-12.

Sevcikova, M., Modra, H., Slaninova, A., Svobodova, Z. (2011). Metals as a cause of oxidative stress in fish: a review. *Veterinární medicína*, *56*(11), 537-546.

SOUZA, C. A. S. 2000. Urbanização na Amazônia. Belém: UNAMA.

Storey, K.G., (1996). Oxidative stress: animal adaptation in nature. Braz. J. Med. Res., v. 29, p. 1715-1733.

The jamovi project (2023). *jamovi*. (Version 2.4) [Computer Software]. Retrieved from https://www.jamovi.org.

Verlecar XN, Jena KB, Chainy GBN (2008). Modulação das defesas antioxidantes na glândula digestiva de *Perna viridis* (L.), em exposições ao mercúrio. Quimosfera 71:1977–1985

Viana, L. F., Kummrow, F., Cardoso, C. A. L., de Lima, N. A., Solórzano, J. C. J., do Amaral Crispim, B., ... & Florentino, A. C. (2021). High concentrations of metals in the waters from Araguari River lower section (Amazon biome): Relationship with land use and cover, ecotoxicological effects and risks to aquatic biota. *Chemosphere*, *285*, 131451.

Viarengo, A., Ponzano, E., Dondero, F., Fabbri, R. (1997). A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. *Marine environmental research*, *44*(1), 69-84.

Watson, G. J., Pini, J. M., & Richir, J., (2018). Chronic exposure to copper and zinc induces DNA damage in the polychaete Alitta virens and the implications for future toxicity of coastal sites. *Environmental Pollution*, *243*, 1498-1508.

White CC, Viernes H, Krejsa CM, Botta D, Kavanagh T.J., (2003). Fluorescence-based microtiter plate assay for glutamate-cysteine ligase activity. Anal Biochemi 318:175–180. https:// doi. org/ 10. 1016/ S0003-2697(03) 00143-X

Wilczek, G., Babczyńska, A., Augustyniak, M., Migula, P., (2004). Relations between metals (Zn, Pb, Cd and Cu) and glutathione-dependent detoxifying enzymes in spiders from a heavy metal pollution gradient. *Environmental Pollution*, *132*(3), 453-461.

4. **Considerações Finais**

Os dados obtidos neste estudo revelam que as múltiplas atividades antrópicas que se desenvolveram na região da Amazônia Legal ao longo de décadas estão contribuindo diretamente para a deterioração desses ecossistemas, uma vez que altas concentrações de metais foram verificadas tanto no sedimento quanto na água. As Avaliações de Risco Ecológico (ARAs), por sua vez, evidenciaram uma situação alarmante com alto risco para a biota aquática em relação a diversos metais.

A espécie de ostracode aqui descrita pertence ao gênero *Strandesia*, possivelmente indicando uma convergência evolutiva entre *Neostrandesia striata* e *Bradleytriebella lineata*. Por outro lado, a espécie apresenta excelente potencial para futuros estudos ecotoxicológicos, pois demonstrou rápida adaptabilidade, crescimento e reprodução em laboratório.

A sensibilidade do ostracode *S. rondoniensis* aos metais foi alta e próxima dos valores relatados para outras espécies do mesmo grupo. Dentre os metais avaliados, o Cd se destacou como o mais tóxico, seguido pelo Hg, Cu e Zn. Para misturas metálicas foram identificados diferentes efeitos, com predomínio do sinergismo quando o cobre estava presente em altas concentrações.

Por outro lado, este estudo mostrou que concentrações subletais causaram aumento na quantidade de ERO nas células, e os sistemas de defesa antioxidante, tanto enzimáticos quanto não enzimáticos (SOD, GSH, MT) foram ativados principalmente para os metais Zn, Cd e Hg. No entanto, estes sistemas foram incapazes de conter os efeitos dos metais, resultando no aumento da peroxidação lipídica (LPO) e danos no DNA. Assim, este estudo serve como base sólida para o desenvolvimento de novas políticas públicas de monitoramento e recuperação ambiental no bioma Amazônia, visando a preservação mais adequada da biodiversidade presente neste ecossistema.

4. Final considerations

The data obtained in this study reveal that the multiple human activities that have developed in the Legal Amazon region over decades are directly contributing to the deterioration of this biome, since high concentrations of metals were found in both the sediment and water. The Ecological Risk Assessments (ARAs), in turn, highlighted an alarming situation with a high risk for aquatic biota in relation to several metals.

The ostracod species described here belongs to the genus *Strandesia*, possibly indicating an evolutionary convergence between *Neostrandesia striata* and *Bradleytriebella lineata*. On the other hand, the species presents excellent potential for future ecotoxicological studies, as it has demonstrated rapid adaptability, growth and reproduction in the laboratory.

The sensitivity of *S. rondoniensis* to metals was high and close to values reported for other ostracod species. Among the metals evaluated, Cd was the most toxic, followed by Hg, Cu and Zn. For metallic mixtures, different effects were identified, with a predominance of synergism when copper was present in high concentrations.

On the other hand, this study showed that sublethal concentrations caused an increase in the amount of ROS in cells, and the antioxidant defense systems, both enzymatic and non-enzymatic (SOD, GSH, MT) were activated mainly for the metals Zn, Cd and Hg. However, these systems were unable to contain the effects of metals, resulting in increased lipid peroxidation (LPO) and DNA damage. Thus, this study serves as a solid basis for the development of new public policies for monitoring and environmental recovery in the Amazon biome, aiming to more adequately preserve the biodiversity present in this ecosystem.

Supplementary Material - Chapter 1

Ecological risk assessment for metals in sediment and waters from the Brazilian Amazon region

Table S1 – Metal concentrations in water samples from several freshwater environments monitored in the legal Amazon. Values in red were above the standards established by the National Environment Council – CONAMA (Resolution 357) from Brazil (Brazil, 2005), numbers in red represent values that were above those recommended by law.

State	City	Site	MEC water (mg L^{-1})										Authors
			Co	Mn	Cr	Cu	Ni	Pb	Ba	Zn	Cd	As	
Rondônia	Porto Velho	Urban creeks	0.0009	0.0246	0.005	0.0036	-	0.003	-	0.0103	-	-	Dos Santos (2009)
Amazonas	Manaus	Negro river	-	0.08	-	0.22	0.24	0.22	-	0.04	0.18	-	Pinto *et al.* (2009)
	Manaus	Educandos' basin	9.53	-	0.19	0.62	2.55	3.81	-	-	7.66	-	Da Silva (2010)
	Manaus	Tarumã-Açu basin	-	-	0.11	0.08	-	-	-	0.05	5	-	Santana e Barroncas (2007)
Pará	Santarém	Santarém metropolitan region	4.0E-06	0.006144	0.000112	0.000533	6.70E-05	0.000438	0.00216	0.009737	-	3.90E-05	Morgado (2019)
	Santarém	Tapajós river	-	0.01202	-	-	-	-	0.00247	-	-	-	Miranda *et al.* (2009)
	Igarapé-açu	Cumaru basin headspring	-	-	-	0.047	-	0.0054	-	-	-	-	Felizzola *et al.* (2019)
Amapá	Santana	Amazonas river	0.019	0.02	-	0.006	-	-	-	0.016	0.001	0.722	da Silva Furtado (2016)
	Lourenço	Cassiporé river	-	-	2.69	0.37	-	1.18	-	0.139	0.164	-	Lima *et al.* (2015)
Roraima	Boa Vista	Rio Branco basin	-	0.04	-	-	-	-	0.04	-	-	-	da Silva *et al.* (2014)
Maranhão	São Luis	São Marcos bay	-	0.05	-	-	-	-	-	-	-	-	Sousa (2009)
Tocantins	Ribamar Siqueira	Sono and Araguaia rivers			0.0146	0.0128		0.0138			0.0003		Duarte (2013)
Mato Grosso	Vera	Caiabi river	-	0.0025	-	0.016	0.0015	-	-	0.001	-	-	Eidt (2015)
Water standard from CONAMA 357			0.05	0.1	0.05	0.009	0.03	0.01	0.7	0.18	0.001	0.01	Brasil (2005)

Table S2 – Metal concentrations in sediment samples from several freshwater environments monitored in the legal Amazon. Values in red were above the standards established by the National Environment Council – CONAMA (resolution 454) from Brazil (Brazil, 2012), numbers in red represent values that were above those recommended by law.

State	City	Site	MEC sediment (mg Kg^{-1} dry weight)										Authors
			Co	Mn	Cr	Cu	Ni	Pb	Ba	Zn	Cd	As	
Rondônia	Porto Velho	Tanques creek	1.43	41.02	73.8	33.4	-	19	-	129.1	0.64		Dos Santos *et al.* (2012)
	Ji-Paraná	Jaru Biological Reserve	2.3	61.1	6.5	6	-	5.8	-	13.1	-	-	Assis *et al*. (2018)
	Porto Velho	Madeira river	52	409	52	27	3.8	24	-	101	-	-	Lacerda *et al.* (1990)
	Porto Velho	Madeira river tributary	11.87	-	9.162	19.23	19.78	15.28	-	58.53	-	-	Santos *et al.* (2015)
Amazonas	Humaitá	Puruzinho lake	5.4	63.6	11.8	11.1	-	11.9	-	66.6	-	-	Carvalho *et al.* (2018)
	Manaus	Tarumã-Açu basin	160.3	362.56	63.45	537	152.25	221.6	-	117.1	-	-	Santana e Barroncas (2007)
	Humaitá	Humaitá urban streams	-	-	17.5	-	1.9	12.5	-	-	0.1	2.8	Oliveira *et al.* (2016)
	Manaus	Amazonas river	20	0.06	66	34	27	82	309	109	-	17	Siqueira. Braga e Aprile (2005)
Pará	Belém	Guajará bay	-	-	53.75	11.87	-	-	-	-	3.9	-	Santos (2018)
	Belem	Maguari river	-	-	22.5	23	12	33	67	62.5	-	-	Guimarães *et al*. (2022)
	Belém	Água Preta and Bolonha lake	11.9	-	143.3	23.17	51.83	33.25	-	78.43	0.19	-	Oliveira *et al.* (2018)
	Santarém	Santarém metropolitan region	18.86	52.11	99.63	42.26	3.81	5.48	72.01	66	-	-	Morgado (2019)
	Curuçá	Amazonas estuary	13.9	-	98.9	6.9	24.1	-	-	-	5.4	-	Silva *et al.* (2018)
Acre	Purus	Acre river	-	1139	15.14	33.87	28.65	20.19	-	91.31	3.48	-	Duarte e GIoda (2014)
Amapá	Porto Grande	Amapari river	-	1856.8	86.01	46.13	61.98	44.48	-	72.5	-	-	Da Silva *et al.* (2013)
Maranhão	Cover 83 municipalities	São Marcos bay	-	221.02	7.21	2.2	3.72	4.26	-	10.99	-	-	Santos (2018)
Tocantins	Formoso do Araguaia	Formoso river	-	100	60	20	-	16	40	40	-	-	Guarda *et al.* (2021)
Mato grosso	Pantanal de Poconé	Bento Gomes river	-	315.145	9.16	9.42	6.44	-	-	18.89	-	-	Coringa *et al.* (2016)
Sediment standard from CONAMA 454			-	-	37.9	35.7	18	35	-	123	0.6	5.9	Brasil (2012)

Table S3 – Values of lethal concentrations and references used for the construction of SSD curves.

Metal	LC_{50}	Specie	Group	Authors
Cobalt	*0.016*	*Hyalella azteca*	Crustacean	Borgmann *et al*. (2005)
	1.32	*Daphnia hyalina*	Crustacean	Borgmann *et al*. (2005)
	2.025	*Daphnia pulicaria*	Crustacean	Lind; Alto; Chatterton. (1978)
	2.347	*Ceriodaphnia dubia*	Crustacean	Diamond *et al*. (1992)
	4	*Eudiaptomus padanus*	Crustacean	Baudouin e Scoppa. (1974)
	4.4	*Daphnia magna*	Crustacean	Stephan (1978)
	6.093	*Tisbe holothuriae*	Crustacean	Miliou *et al*. (2000)
	9.72	*Daphnia pulex*	Crustacean	Griffitt *et al*. (2008)
	10	*Danio rerio*	Fish	Griffitt *et al*. (2008)
	16	*Ephemerella subvaria*	Insect	Warnick e Bell. (1969)
	22.7	*Palaemon serratus*	Crustacean	Amiard (1976)
	25.49	*Cypris subglobosa*	Crustacean	Khangarot e Das (2009)
	32.2	*Pimephales promelas*	Fish	Curtis e Ward (1981
	56.87	*Chironomus tentans*	Insect	Khangarot e Ray (1989)
	100	*Lumbriculus variegatus*	Annelid	Ewell *et al*. (1986)
	112.8	*Capoeta fusca*	Fish	Pourkhabbaz *et al*. (2011)
	132.62	*Branchiura sowerbyi*	Annelid	Das e Kaviraj (1994)
	139.32	*Tubifex tubifex*	Annelid	Khangarot (1991)
Cooper	0.008	*Fluminicola virens*	Molluscs	Nebeker *et al*. (1986)
	0.015	*Juga plicifera*	Molluscs	Nebeker *et al*. (1986)
	0.0166	*Daphnia magna*	Crustacean	Lewis (1983)
	0.025	*Allonais inaequalis*	Annelid	Gazonato *et al*. (2019)
	0.025	*Hydra viridissima*	Cnidarian	Karntanut e Pascoe (2002)
	0.029	*Ceriodaphnia dubia*	Crustacean	carlson. Nelson. Hammermenister (1986)
	0.031	*Dero furcatus*	Annelid	Gazonato *et al*. (2019)
	0.042	*Hydra vulgaris*	Cnidarian	Karntanut e Pascoe (2002)
	0.06	*Biomphalaria glabrata*	Molluscs	Ballavere e Gorbi (1981)
	0.093	*Tubifex tubifex*	Annelid	Rathore e Khangarot (2002)
	0.15	*Pimephales promelas*	Fish	Curtis. Copeland. Ward (1978)
	0.22	*Lumbriculus variegatus*	Annelid	Meyer *et al*. (2002)
	0.42	*Limnodrilus hoffmeisteri*	Annelid	Wurtz e Bridges (1961)
	0.608	*Chironomus tentans*	Insect	Nebeker. Cairns. Wise (1984)
	1.2	*Chironomus riparius*	Insect	Taylor. Maund. Pascoe (1991)
	2.05	*Chironomus plumosus*	Insect	Hooftman *et al.* (1989)
	5.79	*Heterocypris incongruens*	Crustacean	Janssen e Persoone (2011)

(to be continued)

Metal	LC_{50}	Specie	Group	Authors
Zinc	0.163	*Ceriodaphnia dubia*	Crustacean	Balanger e Cherry (1990)
	0.21	*Daphnia magna*	Crustacean	Erten *et al*. (1998)
	0.75	*Chironomus tentans*	Insect	Siblay *et al.* (1996)
	0.96	*Nassarius obsoletus*	Molluscs	MacInnes e Thurberg (1973)
	1.82	*Neanthes arenaceodentata*	Annelid	Reish *et al*. (1977)
	3.8	*Radix luteola*	Molluscs	Khangarot e Ray (1987)
	5.48	*Girardia tigrina*	Molluscs	See (1976)
	5.6	*Lymnaea stagnalis*	Molluscs	Guth. Blankespoor. Cairns (1977)
	7	*Perna viridis*	Molluscs	Govindarajan *et al*. (1993)
	8.1	*Lumbriculus variegatus*	Annelid	Bailey. Liu (1980)
	18.1	*Aeolosoma headleyi*	Molluscs	Cairns *et al*. (1978)
	80	*Chironomus sp.*	Insect	Qureshi *et al*. (1980)
	85.04	*Cypris subglobosa*	Crustacean	Khangarot e Das (2009)
	94.3	*Chironomus riparius*	Insect	Ibrahim *et al*. (1998)
	130	*Tubifex tubifex*	Annelid	Qureshi. Saksena. Singh (1980)
	105	*Danio rerio*	Fish	Cairns. Scheier. Loos (1965)
	293.78	*Parreysia cylindrica*	Molluscs	Fugare *et al*. (2004)
Chromium	0.045	*Ceriodaphnia reticulata*	Crustacean	Mount *et al.* (1984)
	0.048	*Daphnia pulex*	Crustacean	Mount *et al.* (1984)
	0.145	*Ceriodaphnia dúbia*	Crustacean	Baral *et al*. (2006)
	0.66	*Physa integra*	Molluscs	Cairns *et al*. (1976)
	0.8	*Daphnia magna*	Crustacean	Cabejszek. Stasiak (1960)
	1.018	*Macrobrachium rude*	Crustacean	Vijayaraman. Geraldine (1992)
	1.187	*Anodonta imbecillis*	Molluscs	Keller. Zam (1991)
	3.16	*Tubifex tubifex*	Annelid	Fargasova (1994)
	12.1	*Aeolosoma headleyi*	Annelid	Cairns *et al.* (1978)
	17.3	*Chironomus sp.*	Insect	Larrain *et al*. (1997)
	22.464	*Pimephales promelas*	Fish	Baral *et a*l. (2006)
	25.3	*Lumbriculus variegatus*	Annelid	Bailey. Liu (1980)
	66.2	*Biomphalaria glabrata*	Molluscs	Bellavere. Gorbi (1981)
	67	*Danio rerio*	Fish	Bellavere. Gorbi (1981)
	69.72	*Caenorhabditis elegans*	Nematode	Chu. Chow (2002)
	195	*Micropterus salmoides*	Fish	Fromm. Schiffman (1958)
	410	*Lepomis macrochirus*	Fish	Turnbull. Demann. Weston (1954)

(to be continued)

Metal	LC_{50}	Specie	Group	Authors
Nickel	0.082	*Tubifex tubifex*	Annelid	Brkovic-Popovic. Popovic (1977)
	0.183	*Ceriodaphnia dúbia*	Crustacean	Schamphelaere *et al.* (2006)
	0.98	*Daphnia pulicaria*	Crustacean	Lind. Alto. Chatterton (1978)
	1.6	*Daphnia magna*	Crustacean	Traudt *et al.* (2016)
	1.7	*Radix luteola*	Molluscs	Khangarot. Ray (1988)
	1.9	*Daphnia hyalina*	Crustacean	Baudouin. Scoppa (1974)
	3.1	*Aphelenchus avenae*	Nematode	Sanchez-Moreno. Camargo. Navas (2006)
	3.3	*Girardia tigrina*	Nematode	See *et al.* (1976)
	4.33	*Lymnaea acuminata*	Molluscs	Khangarot. Mathur. Durve (1982)
	7.91	*Pimephales promelas*	Fish	Pickering. Henderson (1966)
	9.56	*Poecilia reticulata*	Fish	Pickering. Henderson (1966)
	15.9	*Lepomis macrochirus*	Fish	Pickering. Henderson (1966)
	17	*Cephalobus persegnis*	Nematode	Sanchez-Moreno. Camargo. Navas (2006)
	24.815	*Viviparus bengalensis*	Molluscs	Gupta. Khangarot. Durve (1981)
	39.17	*Stenocypris major*	Crustacean	Shuhaimi-Othman *et al*. (2011)
	56.82	*Chironomus javanus*	Insect	Shuhaimi-Othman *et al.* (2011)
	81.3	*Chironomus riparius*	Insect	Powlesland. George (1986)
	105	*Panagrellus silusiae*	Nematode	Haight. Mudry. Pasternak (1982)
Lead	0.28	*Ceriodaphnia dúbia*	Crustacean	Schubauer-Berigan *et al*. (1993)
	0.6	*Daphnia hyalina*	Crustacean	Baudouin. Scoppa (1974)
	2.69	*Daphnia magna*	Crustacean	Khangarot. Ray. Chandra (1987)
	2.8	*Stenocypris major*	Crustacean	Shuhaimi-Othman *et al*. (2011)
	5.5	*Stenocypris malcolmsoni*	Crustacean	Sharma. Selvaraj (1994)
	6.53	*Chironomus javanus*	Insect	Shuhaimi-Othman *et al*. (2011)
	7.5	*Cephalobus persegnis*	Nematode	Sanchez-Moreno. Camargo. Navas (2006)
	10.4	*Pimephales promelas*	Fish	Pickering. Henderson (1966)
	10.99	*Melanoides tuberculata*	Molluscs	Shuhaimi-Othman. Nur-Amalina. Nadzifah 92012)
	19.4	*Tubifex tubifex*	Annelid	Fargasova (1994)
	24.5	*Poecilia reticulata*	Fish	Pickering. Henderson (1966)
	35.06	*Caenorhabditis elegans*	Annelid	Chu. Chow (2002)
	50.8	*Chironomus tentans*	Insect	Ziegenfuss. Renaudette. Adams (1986)
	62.3	*Chironomus riparius*	Insect	Ha. Choi (2008)
	65	*Elimia livecens*	Molluscs	Cairns *et al.* (1976)
	71	*Hediste diversicolor*	Annelid	Bat *et al*. (2001)
	220	*Chironomus sp.*	Insect	Qureshi *et a*l. (1980)

(to be continued)

Metal	LC_{50}	Specie	Group	Authors
Barium	3.15	*Hyalella azteca*	Crustacean	Borgmann *et al.* (2005)
	42.7	*Oncorhynchus mykiss*	Fish	Birge *et al.* (1985)
	46	*Austropotamobius pallipes*	Crustacean	Boutet. Chaisemartin (1973)
	78	*Orconectes limosus*	Crustacean	Boutet. Chaisemartin (1973)
	150	*Salmo trutta*	Fish	Woodiwiss. Fretwell (1974)
	258	*Echinogammarus berilloni*	Crustacean	Vincent. Penicaut. Debord (1986)
	290	*Rasbora heteromorpha*	Fish	Tooby. Hursey. Alabaster (1975)
	395	*Gammarus pulex*	Crustacean	Vincent. Penicaut. Debord (1986)
	410	*Daphnia magna*	Crustacean	LeBlanc (1980)
	500	*Americamysis bahia*	Crustacean	EPA (1978)
	708	*Streptocephalus proboscideus*	Crustacean	Calleja. Persoone. Geladi (1994)
	870	*Leuciscus idus*	Fish	Juhnke. Luedemann (1978)
	1000	*Fundulus heteroclitus*	Fish	Dorfman (1977)
	3200	*Gambusia affinis*	Fish	Wallen. Greer. Lasater (1957)
Arsenic	1.8	*Ceriodaphnia reticulata*	Crustaceam	Mount. Norberg (1984)
	1.9	*Daphnia pulex*	Crustaceam	Mount. Norberg (1984)
	2.4	*Ceriodaphnia dubia*	Crustaceam	Hu *et al*. (2012)
	3.8	*Daphnia magna*	Crustaceam	EEDB (1992)
	15	*Poecilia latipinna*	Fish	Abdelghani. Anderson. McDonell (1980)
	23.2	*Danio rerio*	Fish	Tisler. Zagorc-Koncan (2002)
	40	*Physella acuta*	Molluscs	Hashimoto. Nishiuchi (1981)
	57	*Philodina roseola*	Rotifer	Schaefer. Pipes (1973)
	69	*Chironomus sp.*	Insect	Jeyasingham. Ling (2000)
	97	*Tanytarsus dissimilis*	Insect	Holcombe. Phipps. Fiandt (1983)
	142	*Polypedilum sp.*	Insect	Jeyasingham. Ling (2000)
	190.54	*Tubifex tubifex*	Annelid	Fargasova (1994)
	395	*Chironomus zealandicus*	Insect	Jeyasingham. Ling (2000)
	644.3	*Pimephales promelas*	Fish	Curtis. Copeland. Ward (1979)
Manganese	0.15	*Canthocamptus sp.*	Crustaceam	Rao. Nath (1983)
	0.42	*Tubifex tubifex*	Annelid	Fargasova (1999(
	0.745	*Nais elinguis*	Annelid	Shuhaimi-Othman *et al*. (2012)
	1.73	*Stenocypris major*	Crustaceam	Shuhaimi-Othman *et a*l. (2011)
	8.82	*Chironomus javanus*	Insect	Shuhaimi-Othman *et al*. (2011)
	16.03	*Microhyla ornata*	Amphibians	Rao. Madhyastha (1987)
	29	*Daphnia magna*	Crustaceam	Stephan (1978)
	32.05	*Daphnia pulex*	Crustaceam	Slabbert. Venter (1999)
	32.81	*Ceriodaphnia dubia*	Crustaceam	Hockett. Mount (1996)
	92	*Duttaphrynus melanostictus*	Amphibians	Shuhaimi-Othman *et al.* (2011)

(to be continued)

Metal	LC_{50}	Specie	Group	Authors
Manganese	120.43	*Melanoides tuberculata*	Molluscs	Shuhaimi-Othman *et al.* (2012)
	470.98	*Tilapia guineensis*	Fish	Oyewo. Don-Pedro (2006)
	4540	*Colisa fasciata*	Fish	Nath. Kumar (1987)
	19154	*Tympanotonus fuscatus*	Molluscs	Oyewo. Don-Pedro (2006)
Cadmium	0.00648	*Periophthalmus waltoni*	Fish	Bu-Olayan e Thomas (2008)
	0.239	*Nais elinguis*	Worms	Shuhaimi-Othman *et al.* (2012)
	0.56	*Cyprinus carpio*	Fish	Alam e Maughan (1995)
	0.64	*Stenocypris major*	Crustacean	Shuhaimi-Othman *et al.* (2011)
	1.65	*Chironomus javanus*	Insect	Shuhaimi-Othman *et al*. (2011)
	12.93	*Daphnia pulex*	Crustacean	Birge *et al*. (1985)
	15	*Daphnia magna*	Crustacean	Dowden e Bennett (1965)
	16	*Hyalella azteca*	Crustacean	Borgmann *et al*. (2005)
	18.5	*Austropotamobius pallipes*	Crustacean	Boutet e Chaisemartin
	21	*Nitocra spinipes*	Crustacean	Dowden e Bennett (1965)
	21.84	*Pimephales promelas*	Fish	Birge *et al.* (1985)
	22	*Orconectes limosus*	Crustacean	Boutet e Chaisemartin
	36.69	*Ceriodaphnia dubia*	Crustacean	Fort e Stover (1995)
	71	*Cherax destructor*	Crustacean	Khan e Nugegoda (2007)
	73.17	*Planorbarius sp.*	Crustacean	Furmanska (1979)
	81.08	*Asellus aquaticus*	Crustacean	Furmanska (1979)
	117.18	*Poecilia reticulata*	Fish	Furmanska (1979)

Table S4: Contamination factor (CF) scale values (Hakanson, 1980).

CF	CF≥6	Hight contamination
	3≤CF<6	Moderate contamination
	1≤CF<3	Considerable contamination
	CF<1	Low contamination

Table S5 – Contamination factor (CF) of heavy metals in sediment samples from different regions of the Amazon.

City	Site	CF - Co	CF - Mn	CF - Cr	CF - Cu	CF - Ni	CF - Pb	CF - Ba	CF - Zn	CF - Cd	CF - As
Porto Velho	Tanques creek	0.7	1.4	0.4	12.4	-	3.5	-	47.8	6.4	-
Machadinho do Oeste	Jaru Biological Reserve	1.1	2.1	0.0	2.2	-	1.1	-	4.9	-	-
Porto Velho	Madeira river	24.8	14.1	0.3	10.0	0.5	4.4	-	37.4	-	-
Porto Velho	Madeira river tributary	5.7	-	0.0	7.1	2.7	2.8	-	21.7	-	-
Humaitá	Puruzinho lake	21439	-	234.4	16643.3	-	8366.7	-	-	-	-
Manaus	Tarumã-Açu basin	76.3	12.5	0.3	198.9	21.1	41.0	-	43.4	-	-
Humaitá	Humaitá urban streams	-	-	0.1	-	0.3	2.3	-		1	0.34
Manaus	Amazonas river	9.5	0.0	0.3	12.6	3.8	15.2	147.1	40.4	-	2.05
Belém	Guajará bay	-	-	0.3	4.4	-	-	-		39	-
Belem	Maguari river	-	-	0.1	8.5	1.7	6.1	31.9	23.1	-	-
Belém	Água Preta and Bolonha lake	5.7	-	0.7	8.6	7.2	6.2	-	29.0	1.9	-
Santarém	Santarém metropolitan region	9.0	1.8	0.5	15.7	0.5	1.0	34.3	24.4	-	-
Nazaré do Mocajuba	Amazonas estuary	6.6	-	0.5	2.6	3.3	-	-	-	54	-
Purus	Acre river	-	39.1	0.1	12.5	4.0	3.7	-	33.8	34.8	-
Porto Grande	Amapari river	-	63.8	0.4	17.1	8.6	8.2	-	26.9	-	-
Cover 83 municipalities	São Marcos bay	-	7.6	0.0	0.8	0.5	0.8	-	4.1	-	-
Araguaia	Formoso river	-	3.4	0.3	7.4	-	3.0	19.0	14.8	-	-
Pantanal de Poconé	Bento Gomes river	-	10.8	0.0	3.5	0.9	-	-	7.0	-	-

Table S6 - Hazard concentrations for 5 (HC_5) and 50% (HC_{50}) of the species (mg L^{-1}) and confidence intervals (95% CI) for all metals analyzed. All curves were constructed based on LC_{50} values for species from different groups of aquatic organisms (Table S3).

Metal	HC_5		HC_{50}	
	Value	CI 95%	Value	CI 95%
Cu	0.0042	0.0009 - 0.012	0.107	0.041 - 0.242
Ni	0.205	0.042 - 0.566	5.63	2.498 - 12.73
Ba	12.37	2.48 - 33.92	225.2	99.63 - 509.14
As	1.112	0.17 - 3.572	31.79	12.40 - 81.51
Cr	0.045	0.0045 - 0.206	5.26	1.582 - 17.51
Pb	0.61	0.145 - 1.591	12.09	5.698 - 25.67
Mn	0.096	0.0041 - 0.684	27.72	5.622 - 136.6
Co	0.317	0.0587 - 0.9888	12.21	4.99 - 29.87
Zn	0.206	0.024 - 0.716	10.68	3.921 - 29.09
Cd	0.05	0.004 - 0.26	5.37	1.47 - 19.56

Figure S1 - PCA analysis with the concentrations of metals in water samples from the Amazon Basin. The PC1 accounted for 46% of all variation, and the PC2 28%. Sampling stations were presented as the municipalities, being PV - Porto Velho (Rondônia State), MA - Manaus (Amazonas State), ST - Santarém (Pará State), AI – Igarapé-Açu (Pará State), SA – Santana (Amapá State), LR – Lourenço (Amapá State), BV – Boa Vista (Roraima State), SL – São Luis (Maranhão State), RS - Ribamar Siqueira (Tocantins State), VR – Vera (Mato Grosso).

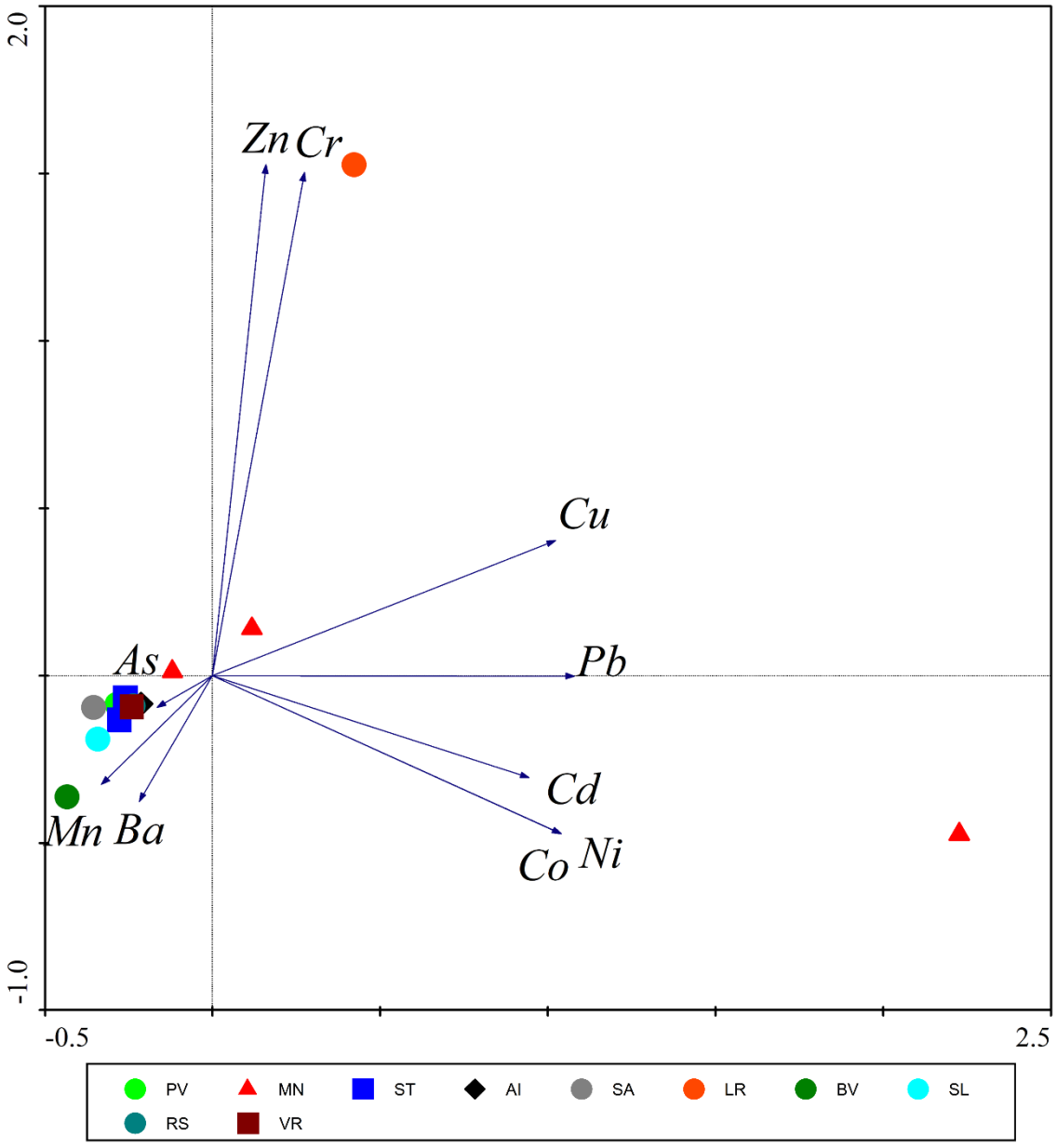


Figure S2 - PCA analysis with the concentration of metals in sediment samples from the Amazon Basin. The PC1 accounted for 42% of all variation, and the PC2 21%. Sampling stations were presented as the municipalities, being PV - Porto Velho (Rondônia State), MO - Machadinho do Oeste (Rondônia State), HU - Humaitá (Amazonas State), MA - Manaus (Amazonas State), BE - Belém (Pará State), ST - Santarém (Pará State), NM - Nazaré do Mocajuba (Pará State), PU - Purus (Acre State), PG - Porto Grande (Amapá State), Araguaia (Tocantins State), PP - Pantanal de Poconé (Mato Grosso State).

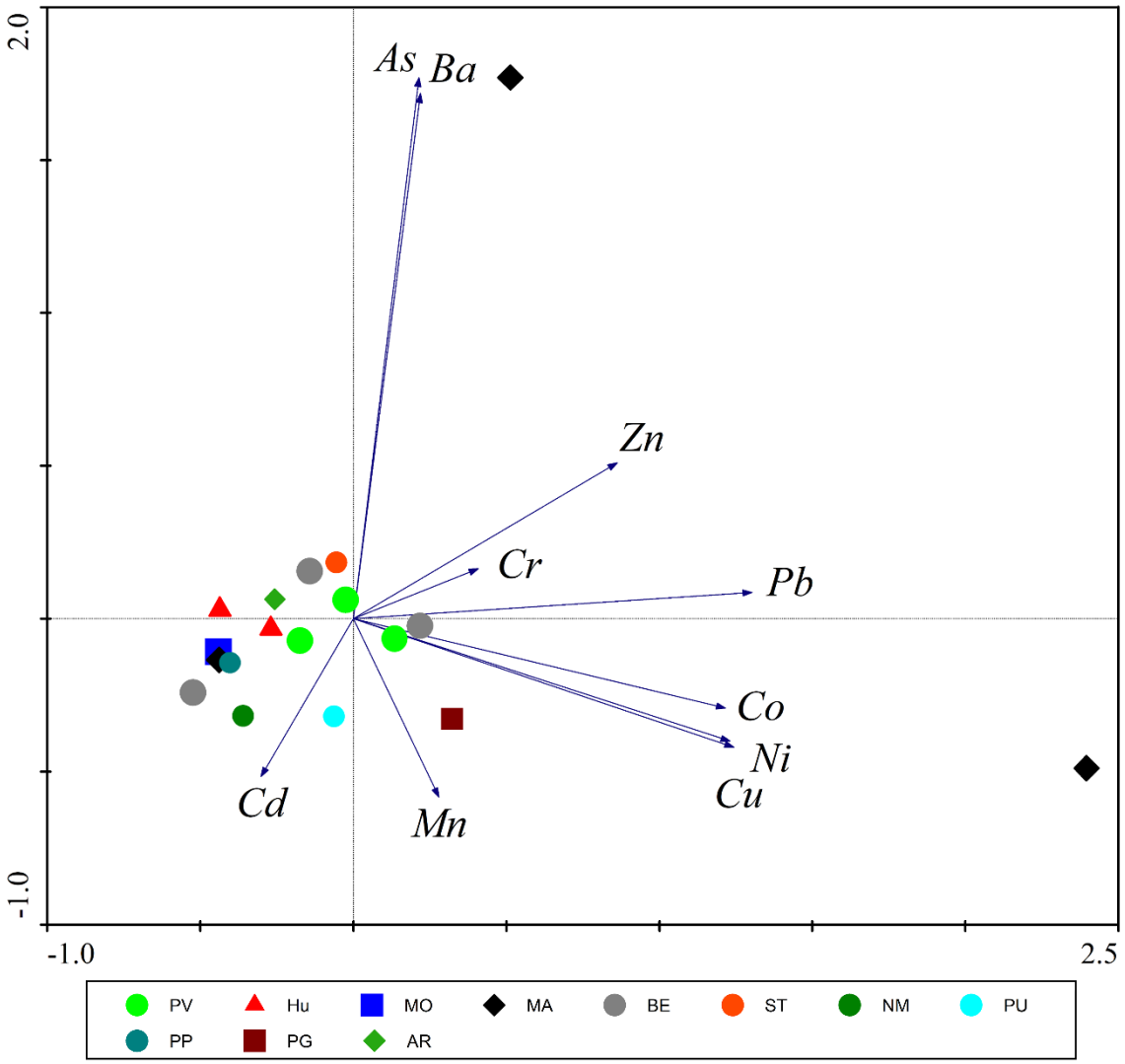


Table S7 – Risk quotient values for metals in water samples in different regions of the Amazon. Values in red denote high risks.

State	City	Site	Risk Quotient									
			Co	Mn	Cr	Cu	Ni	Pb	Ba	Zn	Cd	As
Rondônia	Porto Velho	Urban creeks	0.01420	1.281	0.556	4.3	-	0.025	-	0.25	-	-
Amazonas	Manaus	Negro river	-	4.1667	-	261.9	5.9	1.8	-	0.97	18	-
	Manaus	Educandos' basin	150.3	-	21.111	738.1	62.2	31.2	-	-	766	-
	Manaus	Tarumã-Açu basin	-	-	12.222	95.2	-	-	-	1.21	500	-
Pará	Santarém	Santarém metropolitan region	0.00006	0.320	0.012	0.635	0.002	0.004	0.0009	0.236	-	0.0002
	Santarém	Tapajós river	-	0.626	-	-	-	-	0.0010	-	-	-
	Igarapé-açu	Cumaru basin headspring	-	-	-	56.0	-	0.044	-	-	-	-
Amapá	Santana	Amazonas river	0.30	1.0417	-	71.4	-	-	-	0.39	0.1	3.25
	Lourenço	Cassiporé river	-	-	298.889	440.5	-	9.67	-	3.37	16.4	-
Roraima	Boa Vista	Rio Branco basin	-	2.0833	-	-	-	-	0.0162	-	-	-
Maranhão	São Luis	São Marcos bay	0.789	-	-	-	-	-	-	-	-	-
Tocantins	Ribamar Siqueira	Sono and Araguaia rivers	-	-	1.622	15.2	-	0.113	-	-	0.03	-
Mato Grosso	Vera	Caiabi river	-	0.1302	-	19.0	0.037	-	-	0.024	-	-

Table S8 – Risk quotient values for metals in sediment samples in different regions of the Amazon. Values in yellow denote medium risk and in red high risk.

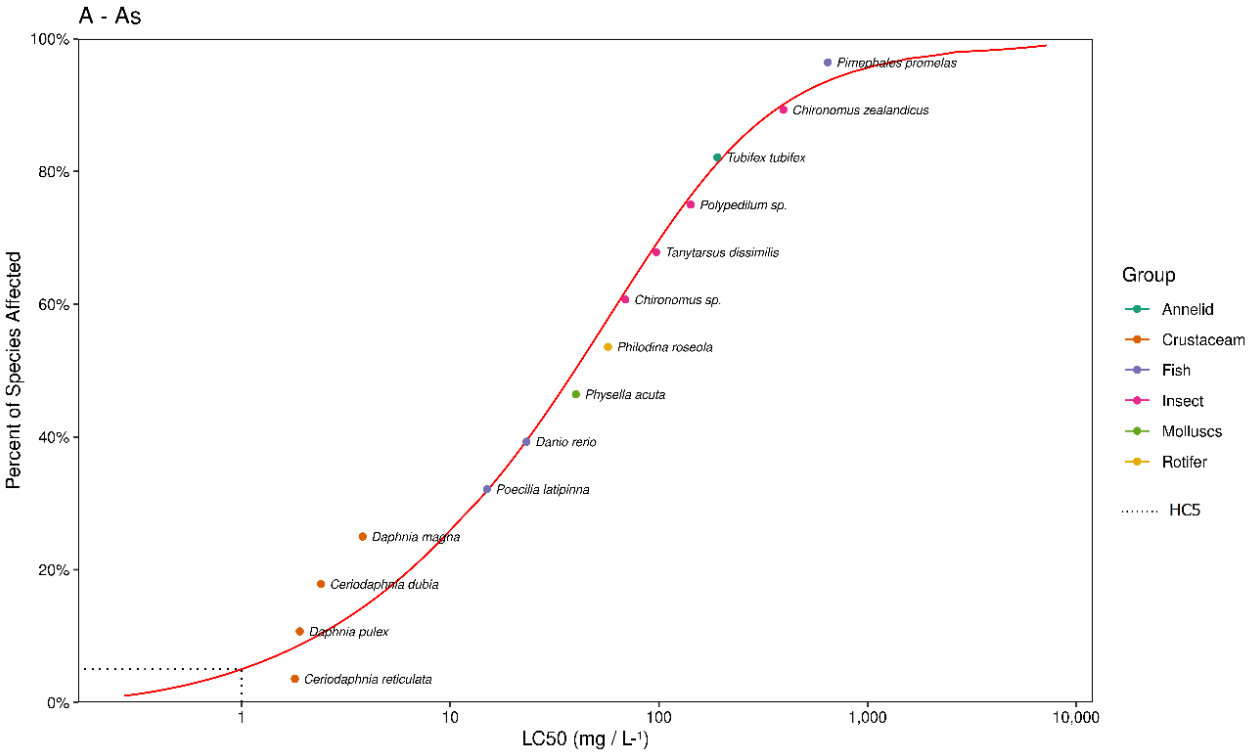
State	City	Site	Risk Quotient									
			Co	Mn	Cr	Cu	Pb	Ba	Zn	Ni	As	Cd
Rondônia	Porto Velho	Tanques creek	0.6	0.1	8.7	0.38	0.22	-	64.44	-	-	0.019
	Ji-Paraná	Jaru Biological Reserve	1.0	0.1	0.8	0.07	0.07	-	6.54	-	0	-
	Porto Velho	Madeira river	23.2	1.0	6.1	0.31	0.28	-	50.42	7.39	-	-
	Porto Velho	Madeira river tributary	5.3	-	1.1	0.22	0.18	-	29.22	38.45	-	0.000
Amazonas	Humaitá	Puruzinho lake	2.4	0.2	1.4	0.13	0.14	-	33.25	-	-	-
	Manaus	Tarumã-Açu basin	71.6	0.9	7.5	6.18	2.57	-	58.45	295.94	-	-
	Humaitá	Humaitá urban streams	-	-	2.1	-	0.15	-	-	3.69	0.64	0.003
	Manaus	Amazonas river	8.9	0.0	7.8	0.39	0.95	14.48	54.41	52.48	3.90	-
Pará	Belém	Guajará bay	-	-	6.3	0.14	-	-	-	-	-	0.116
	Belem	Maguari river	-	-	2.66	0.26	0.38	3.14	31.20	23.325	-	-
	Belém	Água Preta and Bolonha lake	2.81	-	16.92	0.27	0.39	-	39.15	100.74	-	0.006
	Santarém	Santarém metropolitan region	3.60	0.124	11.76	0.49	0.06	3.37	32.95	7.41	-	-
	Curuçá	Amazonas estuary	2.2	-	11.7	0.28	0.06	-	-	46.84	-	0.160
Acre	Purus	Acre river	-	2.70	1.8	0.39	0.23	-	45.58	55.69	-	0.103
Amapá	Porto Grande	Amapari river	-	4.40	10.2	0.53	0.52	-	36.19	120.47	-	2.150
Maranhão	Cover 83 municipalities	São Marcos bay	-	0.52	0.9	0.03	0.05	-	5.49	7.23	-	-
Tocantins	Formoso do Araguaia	Formoso river	-	0.24	7.1	0.23	0.19	1.87	5.49	-	-	1.186
Mato grosso	Pantanal de Poconé	Bento Gomes river	-	0.75	1.1	0.11	-	-	9.43	12.52	-	-

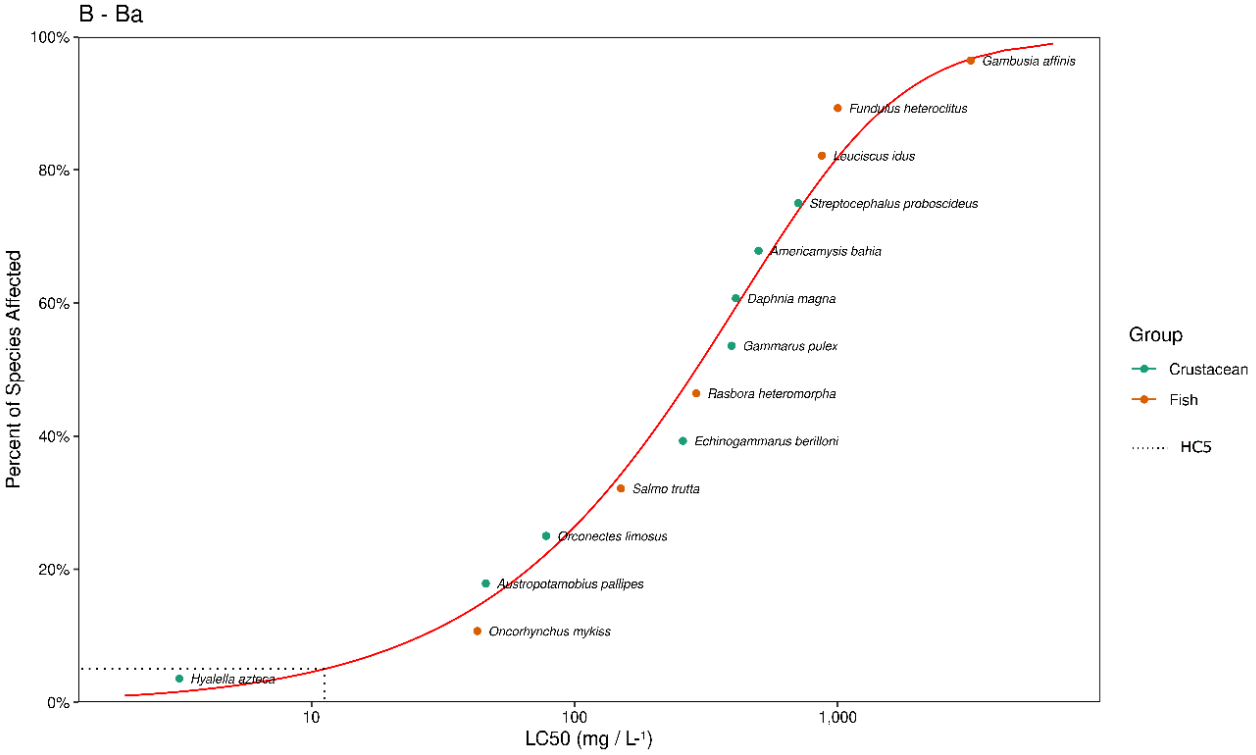
Table S9 - Details of the preparation, digestion, and quantification technique for sediment samples from the different locations analyzed in the Amazon basin.

Preparation and Digestion	Quantification technique	Authors
Dried in an oven at 60ºC for 48h and stored in plastic bags (<5 ºC) through digestion. The samples were digested on a hot plate with 10 mL of 65% HNO_3 for 45 min, 3 mL of 30% H_2O_2 for 15 min, and 6 mL of 37% HCl. The solutions were filtered through quantitative paper (Whatmam 44) and maintained in Teflon tubes. The final volume of 12 mL was adjusted with HCl (0.1N).	Flame atomic absorption spectrophotometry (AAS)	Dos Santos et al. (2012)
Dried in an oven at 60ºC, stored cold, filtered through a 0.075mm mesh, and digested with HNO_3 (65%).	Optical Emission Spectrometry with Plasma-ICP-OES (Perkim-Elmer).	oliveira et al. (2018)
Dried in an oven at 80ºC, filtered (pores <63 µm), and acid digestion (HC1 + HNO_3 + conc. HF.) in Teflon bombs.	Atomic absorption spectrophotometry (AAS)	Lacerda et al. (1990)
Dried in an oven at 80ºC, stored in plastic bags kept cold, and filtered through a 200 µm mesh with digestion with concentrated nitric and hydrochloric acids (HNO_3, HCl).	Flame Atomic Absorption Spectrophotometer	Santos et al. (2015)
Dried in an oven at 40ºC, stored in self-sealing plastic bags, and refrigerated, filtered through a 200 µm mesh, and digested using 65% HNO_3 + 30% H_2O_2 and 37% HCl heated to 80ºC on a hotplate for 16h.	Flame atomic absorption spectrophotometry (AAS - GBC Avanta, Modelo-3000).	Carvalho et al. (2018)
Air-dried for one week, filtered through a 0.053 mm mesh, and digested by HNO_3+HCl (3:1) heated to 105°C.	Atomic absorption spectrometry	Santana e Barroncas, (2007)
Digested according to 6010C (EPA, 2000).	Atomic emission spectrometry with inductively coupled plasma as recommended at 6020A (EPA, 1998).	Oliveira et al. (2016)
Dried in an air circulation oven at 60ºC for 48h, stored in sealed plastic bags, and cooled in a cold chamber at -20 ºC, filtered through a 230 µm mesh deferred in acid attack with $HClO_4$ + HF (1:1) and HCl+HNO_3 (1:1).	Plasma Emission Spectrometry - ICP	Siqueira, braga e Aprile (2005)
Dried at room temperature, stored in plastic bags kept under refrigeration, filtered through a 270mesh mesh, digested in HNO_3 65%+ HCl 38%+HF 40%+ H_3BO_3 99.59% exposed to microwave radiation.	Induced Coupled Plasma Optical Emission Spectrometry (ICP OES)	Santos (2018)
Oven-dried at 60ºC, stored in refrigerated zip-lock plastic bags, filtered through <63 µm mesh, and digested in HCl + HNO_3 (3:1) in a hot block at 85ºC for 2h.	Inductively coupled plasma optical emission spectrometry (ICP-OES, Model 2000, BAIRD, MA, USA)	Guimarães et al. (2022)
Dried in an oven at 80ºC, stored at -20ºC, and filtered through a 200 µm mesh.	Standard Methods for the Examination of Water and Waste Water APHA 2005	Oliveira et al. (2018)
Dried at room temperature, stored in plastic bags in the freezer, filtered through a <63 µm mesh, and digested at 950 ºC for 30 minutes.	Induced Plasma Optical Emission Spectrometry (ICP-OES)	Morgado, (2019)

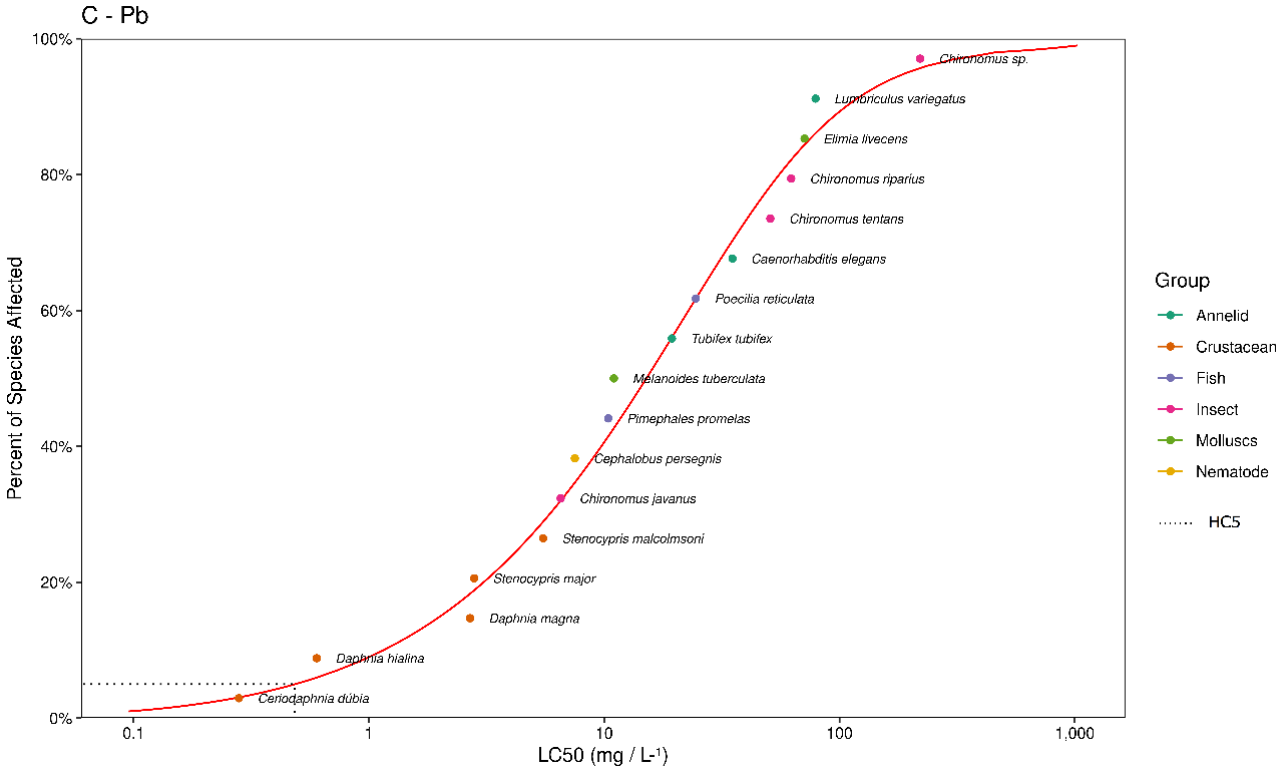
Dried at room temperature, filtered through a 270 μm mesh, and digested by nitric, hydrochloric, hydrofluoric, and boric acid, taken to the microwave.	ICP-MS	Silva et al., 2018
Dried in an oven at 50-60ºC for 15, digested by HNO_3 heated to 80 ºC for 5h, and centrifuged.	Inductively coupled plasma mass spectroscopy (ICP-MS - Elan 6000, Perkin Elmer, USA)	Duarte e Gioda (2014)
Filtered in <62 μm mesh and digested by HF 48% + HCl + HNO_3.	Optical Emission Spectrometry and Inductively Coupled Plasma Mass Spectrometry (ICP-OES and ICP-MS)	da Silva et al, 2013
Dried in an oven at 100 ºC for 1h, stored sealed in polyethylene bags, filtered through a 2.0 μm mesh, and digested with HCl+HNO_3 (3:1) heated in a microwave oven at 175 ºC for 30 min.	Flame Atomic Absorption Spectrometry	Santos, 2018
Dried in an oven at 60ºC for 3 days + 110ºC for 4h, stored in polyethylene bottles and refrigerated, filtered through a 150 μm mesh, digested by HNO_3 + H_2O_2, heated in a microwave following USEPA 3051 and centrifuged.	Microwave Plasma Atomic Emission Spectrometer	Guarda et al. 2021
Dried in an air circulation oven at 45 ºC, stored in hermetic plastic bags kept in refrigeration (4 ºC), filtered through a 0.053 μm mesh, and digested by HNO_3 + H_2O_2 in a digester block at 95 ºC for 2h (2 times).	Inductively Coupled Plasma Source Atomic Emission Spectrometer	Coringa et al, 2016

Figure S3 - Species sensitivity distribution curve (SSD) plotted based on acute effect concentrations (LC_{50}) values regarding data available in the literature (see Table S3). A – As; B – Ba; C – Pb; D – Cu; E – Cr; F – Mn; G – Ni; H – Zn; I – Co**;** J – Cd.

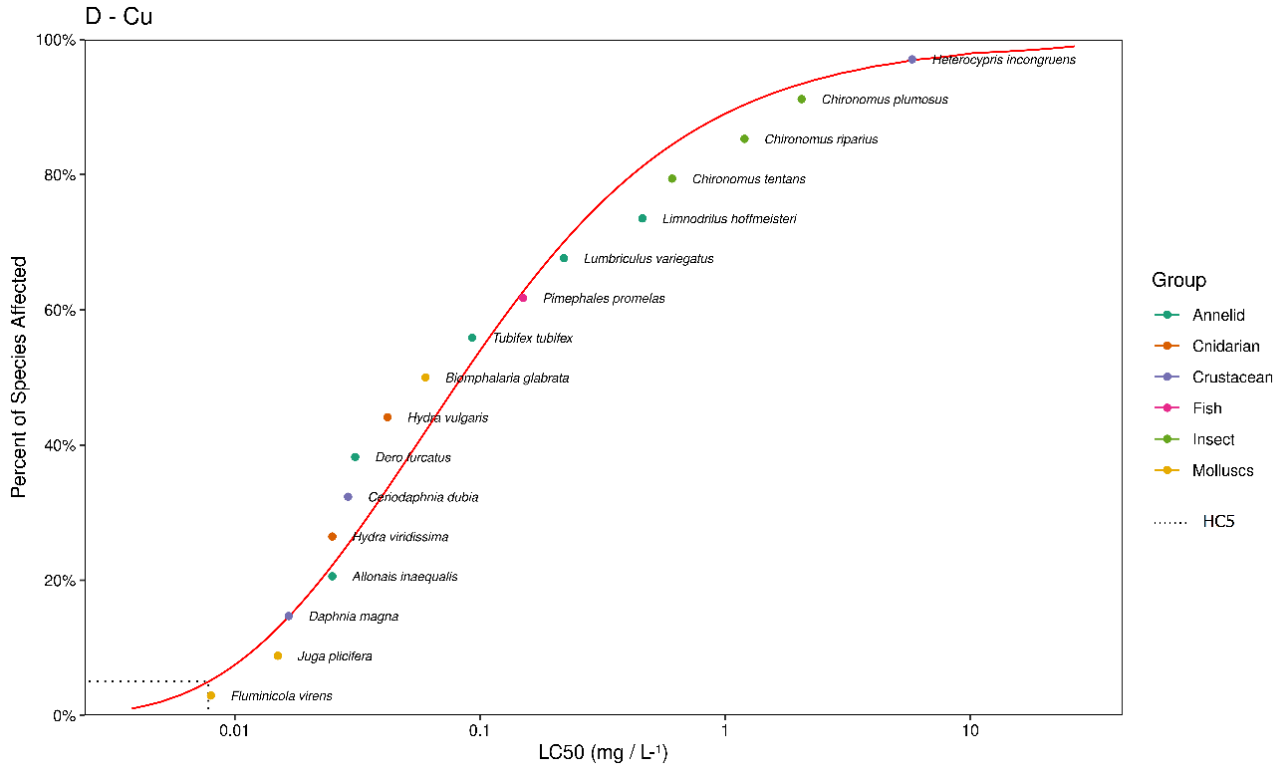




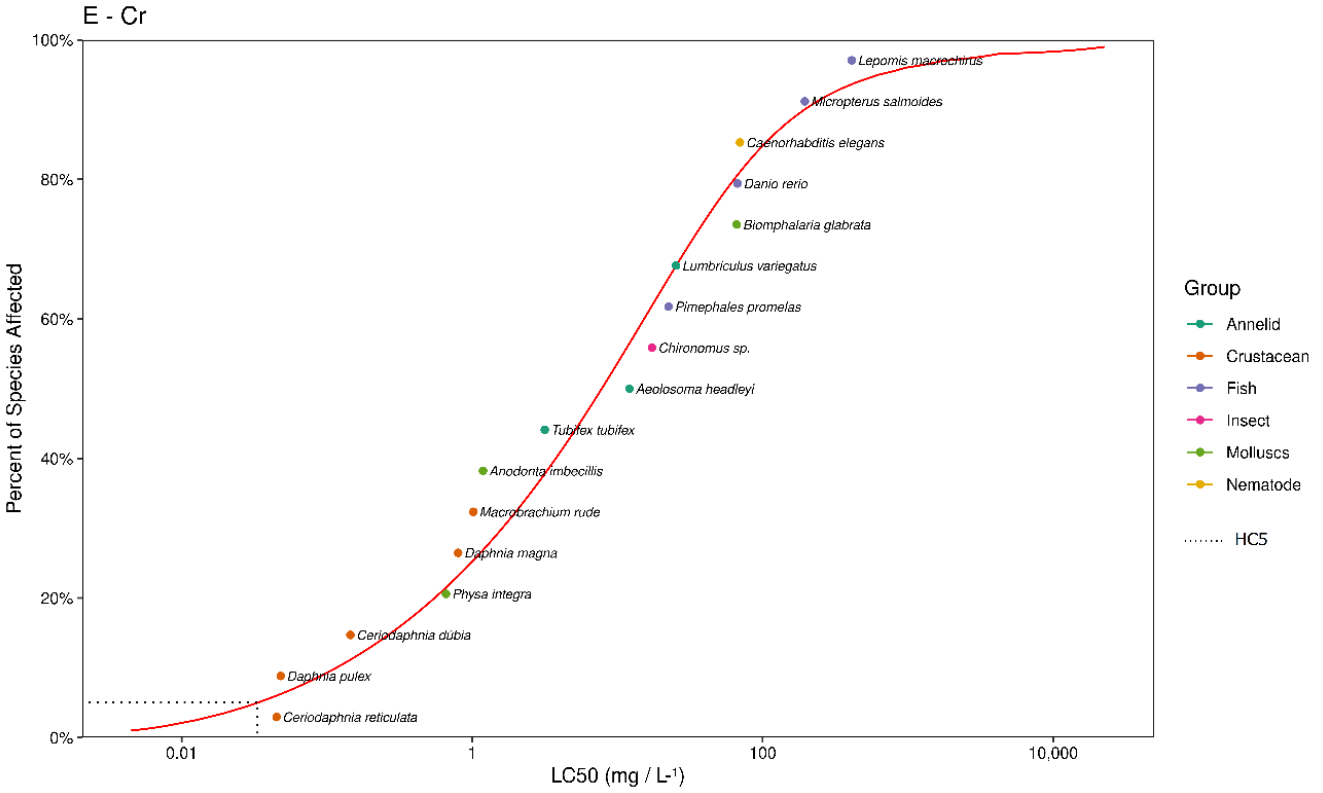
(to be continued)

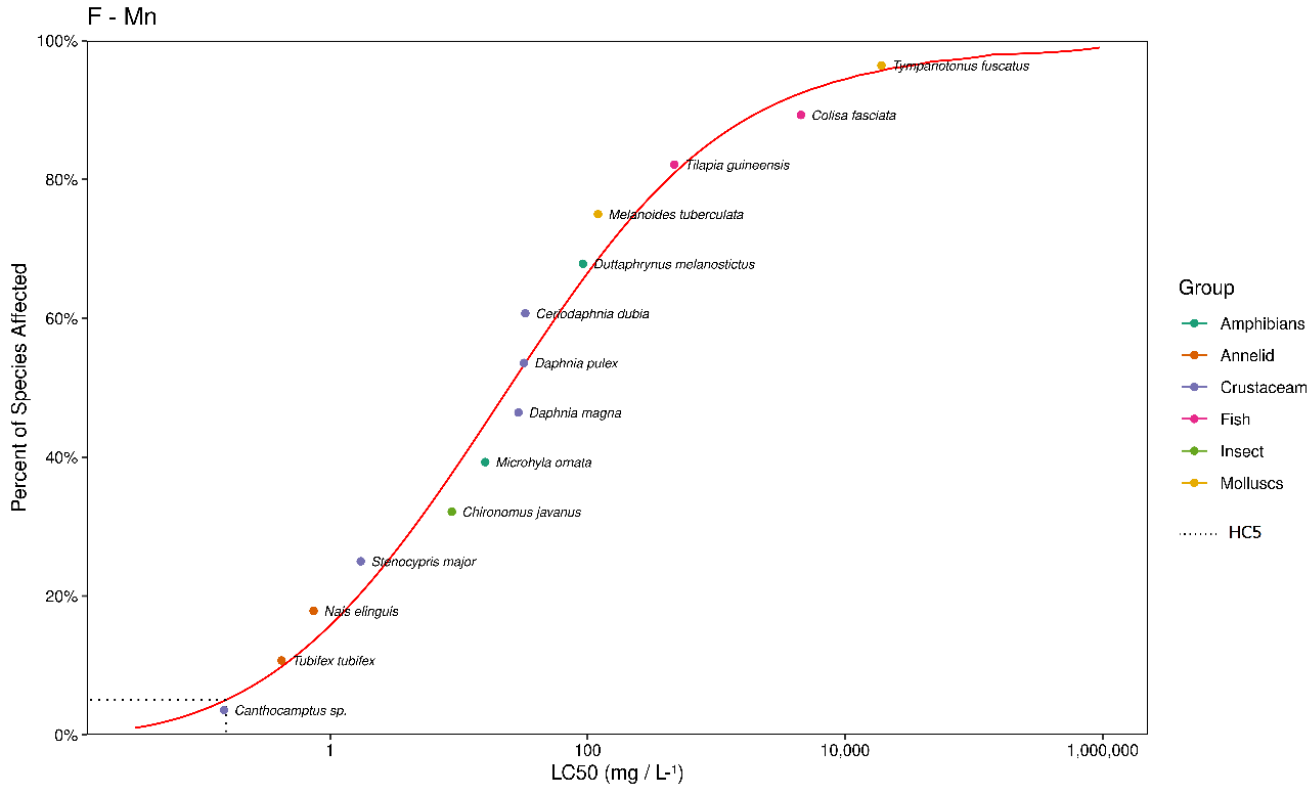


(to be continued)

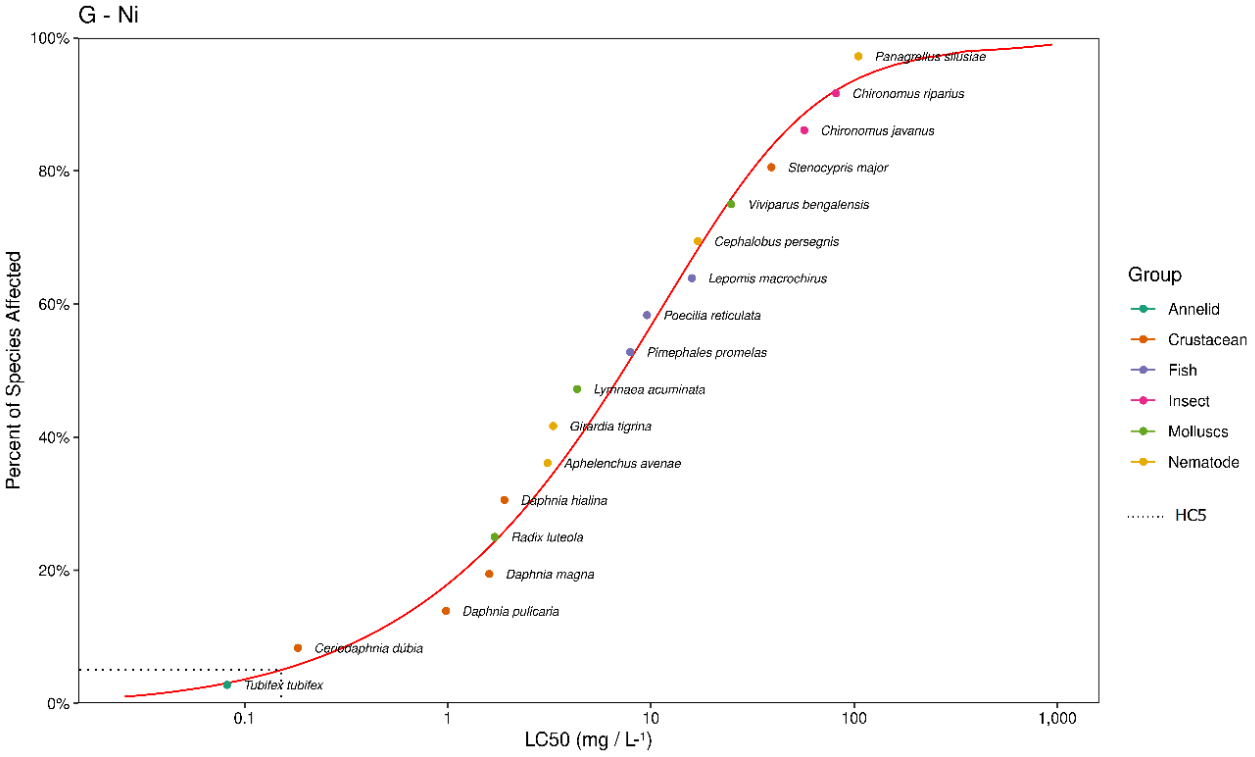


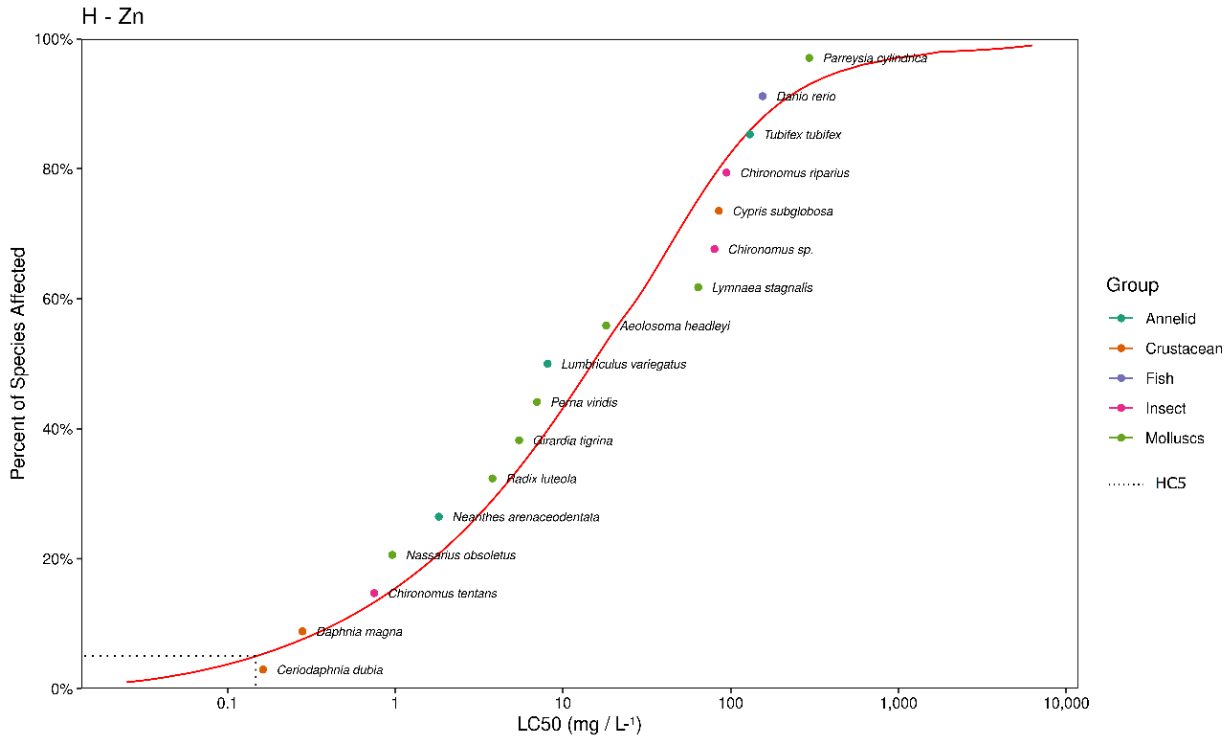
(to be continued)



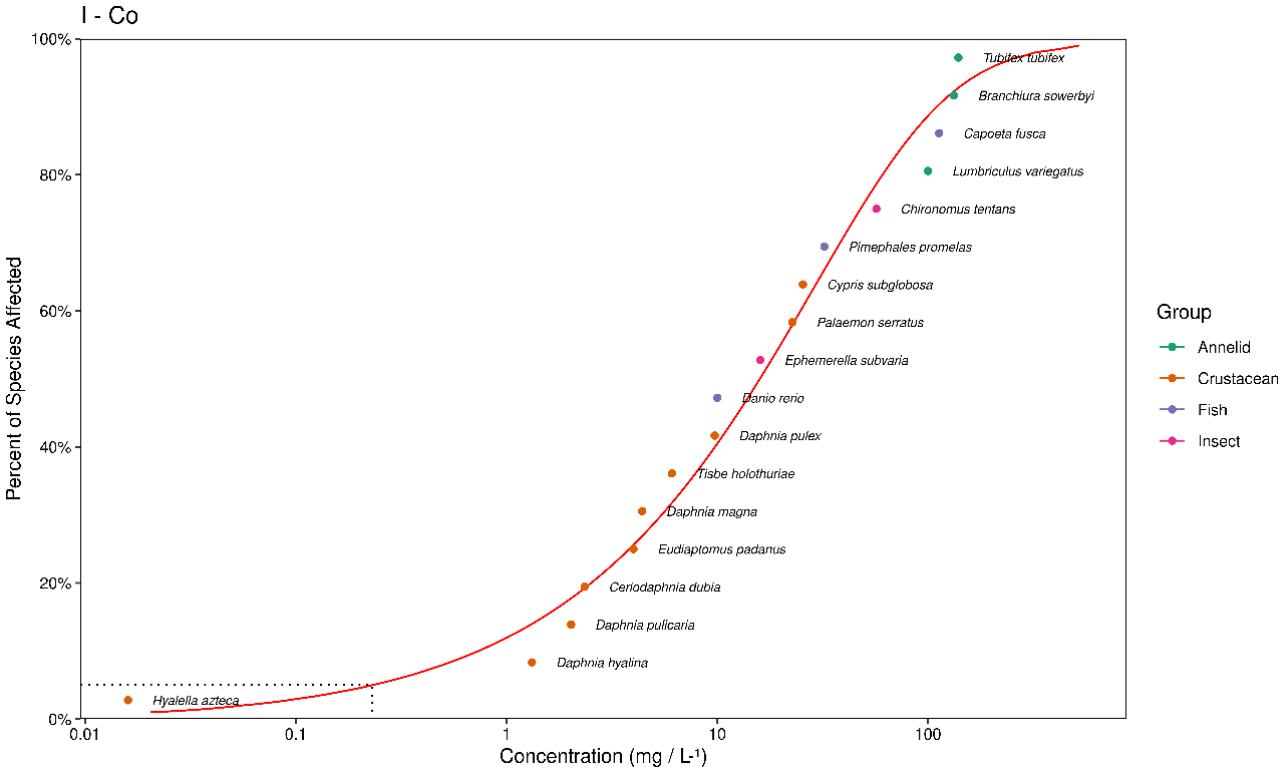


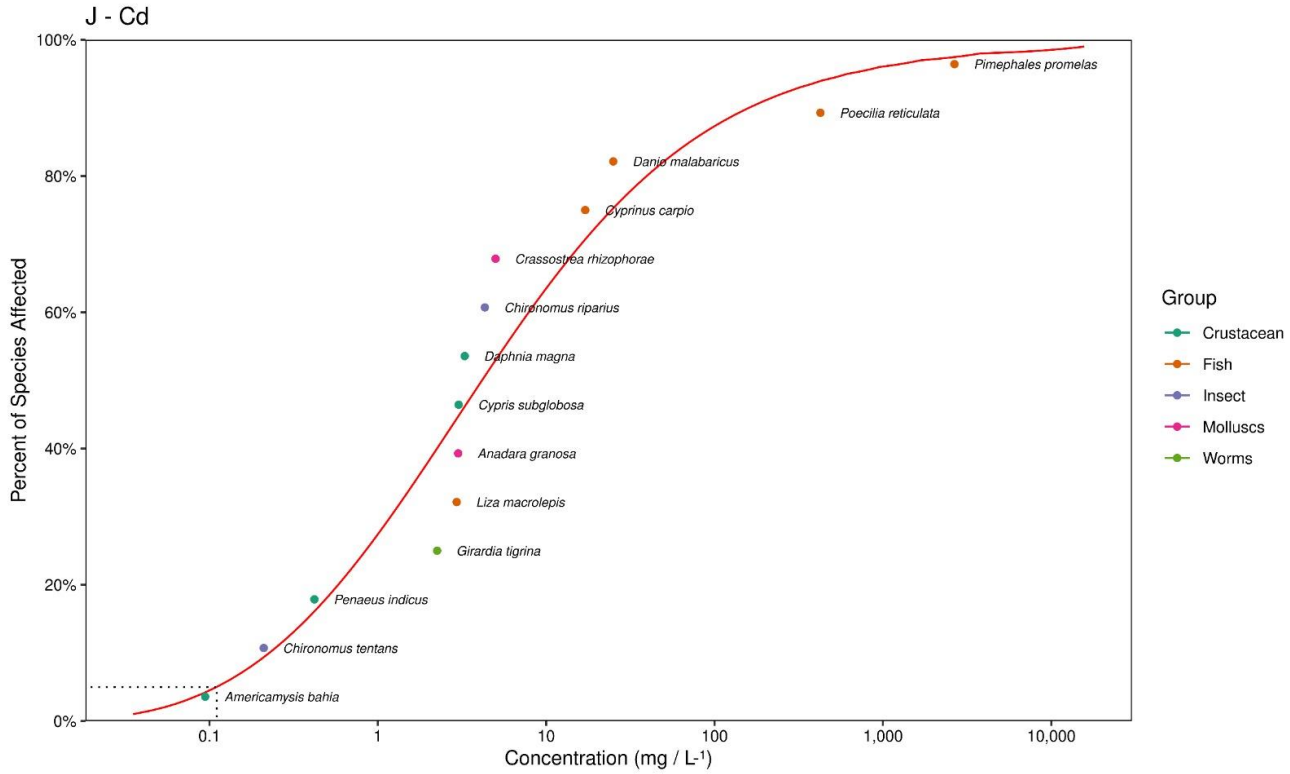
(to be continued)





(to be continued)


I - Co
100%
80%
60%
40%
20%
0%
Percent of Species Affected
0.01
0.1
1
10
100
Concentration (mg / L-1)
Tubifex tubifex
Branchiura sowerbyi
Capoeta fusca
Lumbriculus variegatus
Chironomus tentans
Pimephales promelas
Cypris subglobosa
Palaemon serratus
Ephemerella subvaria
Danio rerio
Daphnia pulex
Tisbe holothuriae
Daphnia magna
Eudiaptomus padanus
Ceriodaphnia dubia
Daphnia pulicaria
Daphnia hyalina
Hyalella azteca
Group
Annelid
Crustacean
Fish
Insect


J - Cd
100%
80%
60%
40%
20%
0%
Percent of Species Affected
0.1
1
10
100
1,000
10,000
Concentration (mg / L-1)
Pimephales promelas
Poecilia reticulata
Danio malabaricus
Cyprinus carpio
Crassostrea rhizophorae
Chironomus riparius
Daphnia magna
Cypris subglobosa
Anadara granosa
Liza macrolepis
Girardia tigrina
Penaeus indicus
Chironomus tentans
Americamysis bahia
Group
Crustacean
Fish
Insect
Molluscs
Worms

References (Table S1)

Brasil. CONAMA – Conselho Nacional do Meio Ambiente., 2005. Resolução CONAMA nº 357/2005 – 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. Conselho Nacional de Meio Ambiente – CONAMA. Brasil.

da Silva Furtado. H.. Florentino. A. C.. Maciel. I. F.. da Silva Costa. J.. & Bezerra. R. M., 2016. Avaliação da acumulação de metais em plantas medicinais da Vila do Elesbão/Santana-AP. *Processos Químicos e Biotecnológicos Volume 4*. 71.

Duarte. R. M., 2013. Estudo de metais pesados na bacia do médio Tocantins. Monografia submetida ao curso de graduação em Engenharia de Alimentos do Centro de Ciências Sociais, Saúde e Tecnologia da Universidade Federal do Maranhão, Maranhão, 65 p.

Eidt. J. I., 2015. Monitoramento da qualidade da água de uma microbacia hidrográfica na região amazônica. Dissertação apresentada ao Programa de PósGraduação em Ciências Ambientais da Universidade Federal de Mato Grosso, Mato Grosso, 75 p.

Felizzola. J. F.. Cak. A. D.. Figueiredo. R. D. O.. & Lima. M. D. O., 2019. Metals and dissolved organic carbon (DOC) of surface waters in two adjacent watersheds in the eastern Amazon. *Revista Ambiente & Água*. *14*.

Lima. D. P. D.. Santos. C.. Silva. R. D. S.. Yoshioka. E. T. O.. & Bezerra. R. M., 2015. Contaminação por metais pesados em peixes e água da bacia do rio Cassiporé. Estado do Amapá. Brasil. *Acta Amazonica*. *45*. 405-414.

Miranda. R. G.. Pereira. S. D. F. P.. Alves. D. T. V.. & Oliveira. G. R. F., 2009. Quality of water resources in the Amazon region-Rio Tapajós: Assessing the case for chemical elements and physical-chemical parameters. *Ambiente e Agua-An Interdisciplinary Journal of Applied Science*. *4*(2). 75-92.

Morgado. M. G. D. A., 2019. Contaminação química de ecossistemas aquáticos e (in) sustentabilidade ambiental na amazônia: estudo de caso na região metropolitana de Santarém, Pará. Tese (doutorado), Universidade de Brasília, Brasília, 136 p.

Pinto. A. G. N.. Horbe. A. M. C.. Silva. M. D. S. R. D.. Miranda. S. A. F.. Pascoaloto. D.. & Santos. H. M. D. C., 2009. Efeitos da ação antrópica sobre a hidrogeoquímica do rio Negro na orla de Manaus/AM. *Acta amazonica*. *39*. 627-638.

Santana. G. P.. & Barroncas. P. D. S. R., 2007. Estudo de metais pesados (Co. Cu. Fe. Cr. Ni. Mn. Pb e Zn) na Bacia do Tarumã-Açu Manaus (AM). *Acta Amazonica*. *37*. 111-118.

Santos. J. P. D., 2009. Avaliação da qualidade da água na rede hídrica superficial de Porto Velho–RO-Brasil. Dissertação (mestrado) Fundação Universidade Federal de Rondônia, Porto Velho, 117 p.

Da Silva. M. B. D., 2010. Análise dos níveis de metais potencialmente tóxicos e análise microbiológica nas águas da bacia do Educandos (Manaus-AM). Dissertação (mestrado), Escola Superior de Ciência da Saúde, Manaus, 67 p.

Silva. J. T. D., 2014. Elementos de Transição nas Águas do Rio Branco e Tributário. RR/BR. In *III Congresso de Iniciação Científica do INPA-CONIC*, Manaus, 124-126.

Sousa. J. K. C., 2009. Avaliação de impactos ambientais causados por metais traço em água. sedimento e material biológico na Baia de São Marcos. Tese (doutorado) Universidade Federal da Paraíba, João Pessoa, 110 p.

References (Table S2 and S10)

Brasil. CONAMA – Conselho Nacional do Meio Ambiente. (2012). Resolução CONAMA nº 454/2012 – Estabelece as diretrizes gerais e os procedimentos referenciais para o gerenciamento do material a ser dragado em águas sob jurisdição nacional. Conselho Nacional de Meio Ambiente – CONAMA. Brasil.

Carvalho. D. P. D.. Almeida. R. D.. Manzatto. Â. G.. Freitas. O. B. D.. & Bastos. W. R., 2018. Dynamics of metals in lacustrine sediments: case study of the Madeira River. Amazon region. *RBRH*. *23*.

Coringa. J. D. E. S.. Pezza. L.. Coringa. E. D. A. O.. & Weber. O. L. D. S., 2016. Distribuição geoquímica e biodisponibilidade de metais traço em sedimentos no Rio Bento Gomes. Poconé-MT. Brasil. *Acta Amazonica*. *46*. 161-174.

da Silva. D. T.. Lafon. J. M.. & Corrêa. J. A. M.. 2013. Distribuição de metais pesados e isótopos de Pb em sedimentos do rio Amapari. setor de Pedra Branca do Amapari–Porto Grande. Amapá. Brasil. *Boletim do Museu Paraense Emílio Goeldi-Ciências Naturais*. *8*(2). 177-203.

Dos Santos. J. P.. de Souza. D. D. O.. Miranda. M. R.. & Bastos. W. R., 2012. Estudos de elementos-traço na cidade de Porto Velho/Rondônia. Amazônia Ocidental. *Ecotoxicology and Environmental Contamination*. *7*(2).

Dos Santos. R. I.. de Almeida Braga. N.. do Nascimento. M. C. N.. & Bastos. W. R., 2015. Metais em sedimentos de fundo na bacia do alto rio Madeira. Bacia Amazônica1, 91-100.

Dos Santos. L. F.. de Sousa. A. M. L.. & Faial. K. D. C. F., 2018. Análise de metais no sedimento na baía do Guajará Belém/PA. *Revista Ibero-Americana de Ciências Ambientais*. *9*(4). 314-325.

De Assis. J. P. M.. Ziemniczak. C. M.. Paulo. J.. Gomes. O.. Júnior. W. A. D. C.. Eduardo. L.. Nascimento. E. L., 2018. Avaliação de metais pesados (zn. cr. pb. cu. mn e co) em amostras de solo e sedimento da reserva biológica do Jaru/RO. *1ª. edição*. 424.

Duarte. A. F.. & Gioda. A., 2014. Inorganic composition of suspended sediments in the Acre River. Amazon Basin. Brazil. *Latin American journal of sedimentology and basin analysis*. *21*(1). 3-15.

Guarda. P. M.. Rambo. M. K. D.. Jaconi. A.. Guarda. E. A.. & da Silva. J. E. C., 2021. Concentrações de metais tóxicos em água. solo e sedimento do rio Formoso. TO. *Research. Society and Development*. *10*(14).

Guimarães. R. H. E.. Wallner-Kersanach. M.. & Correa. J. A. M., 2022. Assessment of anthropogenic metals in shipyard sediment in the Amazon delta estuary in northern Brazil. *Environmental Science and Pollution Research*. *29*(51). 77007-77025.

Lacerda. L. D.. De Paula. F. C. F.. Ovalle. A. R. C.. Pfeiffer. W. C.. & Malm. O., 1990. Trace metals in fluvial sediments of the Madeira River watershed. Amazon. Brazil. *Science of the Total Environment*. *97*. 525-530.

Morgado. M. G. D. A., 2019. Contaminação química de ecossistemas aquáticos e (in) sustentabilidade ambiental na amazônia: estudo de caso na região metropolitana de Santarém, Pará. Tese (doutorado), Universidade de Brasília, Brasília, 136 p.

Oliveira. B. O. S. D.. Tucci. C. A. F.. Neves Júnior. A. F.. & Santos. A. D. A., 2016. Avaliação dos solos e das águas nas áreas de influência de disposição de resíduos sólidos urbanos de Humaitá. Amazonas. *Engenharia Sanitária e Ambiental*. *21*. 593-601.

Oliveira. G. M. T. S. D.. Oliveira. E. S. D.. Santos. M. D. L. S.. Melo. N. F. A. C. D.. & Krag. M. N., 2018. Concentrações de metais pesados nos sedimentos do lago Água Preta (Pará. Brasil). *Engenharia Sanitaria e Ambiental*. *23*. 599-605.

Santana. G. P.. & Barroncas. P. D. S. R., 2007. Estudo de metais pesados (Co. Cu. Fe. Cr. Ni. Mn. Pb e Zn) na Bacia do Tarumã-Açu Manaus (AM). *Acta Amazonica*. *37*. 111-118.

Santos. T. T. L., 2018. Distribuição E Partição Geoquímica De Metais Em Sedimentos De Estuários De Macromaré. Maranhão. Brasil.

Silva. R. G. D.. Santos. R. M.. Lima. M. D. O.. Mendes. L. D. C. D. S.. Mesquita. K. F. C.. & Santos. M. D. L. S., 2018. Trace elements in oyster farming areas in the Amazon.

Siqueira. G. W.. de Lima. W. N.. Mendes. A. S.. Aprile. F. M.. de Santis Braga. E.. & de Lima. E. A. R., 2005. Estudo geoquímico da ocorrência de metais pesados selecionados em sedimentos de fundo da plataforma continental do rio Amazonas–norte do brasil. *Contribuições à Geologia da Amazônia*. *4*. 236-244.

References (Table S3)

Borgmann. U.. Couillard. Y.. Doyle. P.. & Dixon. D. G., 2005. Toxicity of sixty-three metals and metalloids to Hyalella azteca at two levels of water hardness. *Environmental toxicology and chemistry: an international journal*. *24*(3). 641-652.

Lind. D. T.. Alto. K. J.. & Chatterton. S., 1978. *Aquatic Toxicology Study*. Minnesota Environmental Quality Board.

Diamond. J. M.. Winchester. E. L.. Mackler. D. G.. Rasnake. W. J.. Fanelli. J. K.. & Gruber. D., 1992. Toxicity of cobalt to freshwater indicator species as a function of water hardness. *Aquatic toxicology*. *22*(3). 163-179.

Baudouin. M. F.. & Scoppa. P., 1974. Acute toxicity of various metals to freshwater zooplankton. *Bulletin of Environmental Contamination and Toxicology*. *12*. 745-751.

Stephan. C. E., 1978. Chronic Screening Toxicity Test with Daphnia Magna. *Memo to D. Friedman. US EPA*. 3.

Miliou. H.. Verriopoulos. G.. Maroulis. D.. Bouloukos. D.. & Moraitou-Apostolopoulou. M., 2000. Influence of life-history adaptations on the fidelity of laboratory bioassays for the impact of heavy metals (Co2+ and Cr6+) on tolerance and population dynamics of Tisbe holothuriae. *Marine pollution bulletin*. *40*(4). 352-359.

Griffitt. R. J.. Luo. J.. Gao. J.. Bonzongo. J. C.. & Barber. D. S., 2008. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. *Environmental Toxicology and Chemistry: An International Journal*. *27*(9). 1972-1978.

Warnick. S. L.. & Bell. H. L., 1969. The acute toxicity of some heavy metals to different species of aquatic insects. *Journal (Water Pollution Control Federation)*. 280-284.

Amiard. J. C., 1976. Experimental study of acute toxicity of cobalt. antimony. strontium and silver salts in some crustaceans and their larvae and in some teleosteans. *Revue Internationale d'Oceanographie Medicale*. *43*.

Khangarot. B. S.. & Das. S., 2009. Acute toxicity of metals and reference toxicants to a freshwater ostracod. Cypris subglobosa Sowerby. 1840 and correlation to EC50 values of other test models. *Journal of hazardous materials*. *172*(2-3). 641-649.

Curtis. M. W.. & Ward. C. H. , 1981. Aquatic toxicity of forty industrial chemicals: Testing in support of hazardous substance spill prevention regulation. *Journal of Hydrology*. *51*(1-4). 359-367.

Khangarot. B. S.. & Ray. P. K., 1989. Sensitivity of midge larvae of Chironomus tentans Fabricius (Diptera Chironomidae) to heavy metals. *Bull. Environ. Contam. Toxicol.;(United States)*. *42*(3).

Ewell. W. S.. Gorsuch. J. W.. Kringle. R. O.. Robillard. K. A.. & Spiegel. R. C., 1986. Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. *Environmental Toxicology and Chemistry: An International Journal*. *5*(9). 831-840.

Pourkhabbaz. A.. Khazaei. T.. Behravesh. S.. Ebrahimpour. M.. & Pourkhabbaz. H., 2011. Effect of water hardness on the toxicity of cobalt and nickel to a freshwater fish. Capoeta fusca. *Biomedical and Environmental Sciences*. *24*(6). 656-660.

Das. B. K.. & Kaviraj. A., 1994. Individual and interactive lethal toxicity of cadmium. potassium permanganate and cobalt chloride to fish. worm and plankton. *GEOBIOS-JODHPUR-*. *21*. 223-223.

Khangarot. B. S., 1991. Toxicity of metals to a freshwater tubificid worm Tubifex tubifex (Muller). *Bulletin of Environmental Contamination and Toxicology;(United States)*. *46*(6).

Nebeker. A. V.. Stinchfield. A.. Savonen. C.. & Chapman. G. A., 1986. Effects of copper. nickel and zinc on three species of Oregon freshwater snails. *Environmental Toxicology and Chemistry: An International Journal*. *5*(9). 807-811.

Lewis. M. A., 1983. Effect of loading density on the acute toxicities of surfactants. copper. and phenol to Daphnia magna Straus. *Archives of Environmental Contamination and Toxicology*. *12*. 51-55.

Gazonato Neto. A. J.. Moreira. R. A.. Lima. J. C. D. S.. Daam. M. A.. & Rocha. O., 2019. Freshwater neotropical oligochaetes as native test species for the toxicity evaluation of cadmium. mercury and their mixtures. *Ecotoxicology*. *28*. 133-142.

Karntanut. W.. & Pascoe. D., 2002. The toxicity of copper. cadmium and zinc to four different Hydra (Cnidaria: Hydrozoa). *Chemosphere*. *47*(10). 1059-1064.

Carlson. A. R.. Nelson. H.. & Hammermeister. D., 1986. Development and validation of site-specific water quality criteria for copper. *Environmental Toxicology and Chemistry: An International Journal*. *5*(11). 997-1012.

Bellavere. C.. & Gorbi. J., 1981. A comparative analysis of acute toxicity of chromium. copper and cadmium to Daphnia magna. Biomphalaria glabrata. and Brachydanio rerio. *Environmental Technology*. *2*(3). 119-128.

Rathore. R. S.. & Khangarot. B. S., 2002. Effects of temperature on the sensitivity of sludge worm Tubifex tubifex Müller to selected heavy metals. *Ecotoxicology and environmental safety*. *53*(1). 27-36.

Curtis. M. W.. Copeland. T. L.. & Ward. C. H., 1978. Aquatic toxicity of substances proposed for spill prevention regulation. In *National Conference on Control of Hazardous Material Spills Conference Location Miami Beach. FL*.

Meyer. J. S.. Boese. C. J.. & Collyard. S. A., 2002. Whole-body accumulation of copper predicts acute toxicity to an aquatic oligochaete (Lumbriculus variegatus) as pH and calcium are varied. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. *133*(1-2). 99-109.

Wurtz. C. B.. & Bridges. C. H., 1961. Preliminary Results From Macro—Invertebrate Bioassays. In *Proceedings of the Pennsylvania Academy of Science* (pp. 51-56). Pennsylvania Academy of Science.

Nebeker. A. V.. Cairns. M. A.. & Wise. C. M., 1984. Relative sensitivity of Chironomus tentans life stages to copper. *Environmental Toxicology and Chemistry: An International Journal*. *3*(1). 151-158.

Taylor. E. J.. Maund. S. J.. & Pascoe. D., 1991. Toxicity of four common pollutants to the freshwater macroinvertebrates Chironomus riparius Meigen (Insecta: Diptera) and Gammarus pulex (L.)(Crustacea: Amphipoda). *Archives of environmental contamination and toxicology*. *21*. 371-376.

Hooftman. R. N.. Adema. D. M. M.. & Kauffman-Van B. J., 1989. Developing a set of test methods for the toxicological analysis of the pollution degree of water bottoms. Rep. No. 16105. *Netherlands Organization for Applied Scientif ic Research*. *68*.

Janssen. C. R.. & Persoone. G., 2011. International interlaboratory comparison on the subchronic toxicity test with the freshwater ostracod crustacean Heterocypris incongruens. *Report in www. microbiotests. be. section "Publications and Reports*. 1-16.

Erten-Unal. M.. Wixson. B. G.. Gale. N.. & Pitt. J. L., 1998. Evaluation of toxicity. bioavailability and speciation of lead. zinc and cadmium in mine/mill wastewaters. *Chemical Speciation & Bioavailability*. *10*(2). 37-46.

MacInnes. J. R.. & Thurberg. F. P., 1973. Effects of metals on the behaviour and oxygen consumption of the mud snail. *Marine Pollution Bulletin*. *4*(12). 185-186.

Reish. D. J., 1977. *Toxicity of formulated mine tailings on marine polychaeta*. Fisheries and Marine Service.

Khangarot. B. S.. & Ray. P. K., 1987. Zinc sensitivity of a freshwater snail. Lymnaea luteola L.. in relation to seasonal variations in temperature. *Bull. Environ. Contam. Toxicol.;(United States)*. *39*(1).

See. C. L., 1976. *The effect of sublethal concentrations of selected toxicants on the negative phototactic response of Dugesia tigrina* (Doctoral dissertation. Virginia Polytechnic Institute and State University).

Guth. D. J.. Blankespoor. H. D.. & Cairns. J., 1977. Potentiation of zinc stress caused by parasitic infection of snails. *Hydrobiologia*. *55*. 225-229.

Govindarajan. S.. Valsaraj. C. P.. Mohan. R.. Hariprasad. V.. & Ramasubramanian. R., 1993. Toxicity of heavy metals in aquaculture organisms: Penaeus indicus. Perna viridis. Artemia salina and Skeletonema costatum. *Pollut. Res*. *12*(3). 187-189.

Bailey. H. C.. & Liu. D. H. W., 1980. *Lumbriculus variegatus. a benthic oligochaete. as a bioassay organism* (pp. 202-215). ASTM International.

Cairns Jr. J.. Buikema Jr. A. L.. Heath. A. G.. & Parker. B. C., 1978. Effects of temperature on aquatic organism sensitivity to selected chemicals.

Qureshi. S. A.. Saksena. A. B.. & Singh. V. P., 1980. Acute toxicity of four heavy metals to benthic fish food organisms from the River Khan. Ujjain.

Khangarot. B. S.. & Das. S., 2009. Acute toxicity of metals and reference toxicants to a freshwater ostracod. Cypris subglobosa Sowerby. 1840 and correlation to EC50 values of other test models. *Journal of hazardous materials*. *172*(2-3). 641-649.

Ibrahim. H.. Kheir. R.. Helmi. S.. Lewis. J.. & Crane. M., 1998. Effects of organophosphorus. carbamate. pyrethroid and organochlorine pesticides. and a heavy metal on survival and cholinesterase activity of Chironomus riparius Meigen. *Bulletin of Environmental Contamination and Toxicology*. *60*. 448-455.

Caims. J., 1965. A comparison of the sensitivity to certain chemicals of adult zebra danios Brachydanio rerio (Hamilton-Buchanon) and zebra danio eggs with that of adult bluegill sunfish Lepomis macrochirus. *Raf. Notulae Naturale.*. *381*. 1-9.

Fugare. S. H.. Deshmukh. M. P.. Waykar. B. B.. & Pardeshi. B. K., 2004. Acute toxicity of chlorides of zinc. copper and mercury to fresh water bivalve. Parreysia cylindrica(Annandale and Prashad). *Nature. Environment and Pollution Technology*. *3*(2). 147-150.

Mount. D. I.. & Norberg. U. T., 1984. A seven-day life cycle cladoceran toxicity test. *Environmental Toxicology and Chemistry: An International Journal*. *3*(3). 425-434.

Baral. A.. Engelken. R.. Stephens. W.. Farris. J.. & Hannigan. R., 2006. Evaluation of aquatic toxicities of chromium and chromium-containing effluents in reference to chromium electroplating industries. *Archives of Environmental Contamination and Toxicology*. *50*. 496-502.

Cairns Jr. J.. Messenger. D. I.. & Calhoun. W. F., 1976. Invertebrate response to thermal shock following exposure to acutely sub-lethal concentrations of chemicals. *Arch. Hydrobiol.*. *77*(2). 164-175.

Vijayaraman. K.. & Geraldine. P., 1992. Toxicity of cadmium. copper. chromium and zinc to Macrobrachium rude. *Geobios*. *19*. 77-80.

Keller. A. E.. & Zam. S. G., 1991. The acute toxicity of selected metals to the freshwater mussel. Anodonta imbecilis. *Environmental Toxicology and Chemistry: an International Journal*. *10*(4). 539-546.

Fargasova. A., 1994. Toxicity of metals on Daphnia magna and Tubifex tubifex. *Ecotoxicology and environmental safety*. *27*(2). 210-213.

Cairns Jr. J.. Buikema Jr. A. L.. Heath. A. G.. & Parker. B. C., 1978. Effects of temperature on aquatic organism sensitivity to selected chemicals.

Larrain. A.. Riveros. A.. Bay-Schmith. E.. & Roa. R., 1997. Evaluation of three larval instars of the midge Chironomus petiolatus as bioassay tools using a computationally intensive statistical algorithm. *Archives of Environmental Contamination and Toxicology*. *33*. 407-414.

Bailey. H. C.. & Liu. D. H. W., 1980. *Lumbriculus variegatus. a benthic oligochaete. as a bioassay organism* (pp. 202-215). ASTM International.

Bellavere. C.. & Gorbi. J., 1981. A comparative analysis of acute toxicity of chromium. copper and cadmium to Daphnia magna. Biomphalaria glabrata. and Brachydanio rerio. *Environmental Technology*. *2*(3). 119-128.

Chu. K. W.. & Chow. K. L., 2002. Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivative. *Aquatic Toxicology*. *61*(1-2). 53-64.

Fromm. P. O.. & Schiffman. R. H., 1958. Toxic action of hexavalent chromium on largemouth bass. *The Journal of Wildlife Management*. *22*(1). 40-44.

Turnbull. H.. DeMann. J. G.. & Weston. R. F., 1954. Toxicity of various refinery materials to fresh water fish. *Industrial & Engineering Chemistry*. *46*(2). 324-333.

Lind. D. T.. Alto. K. J.. & Chatterton. S., 1978. *Aquatic Toxicology Study*. Minnesota Environmental Quality Board.

Traudt. E. M.. Ranville. J. F.. Smith. S. A.. & Meyer. J. S., 2016. A test of the additivity of acute toxicity of binary-metal mixtures of ni with Cd. Cu. and Zn to Daphnia magna. using the inflection point of the concentration–response curves. *Environmental Toxicology and Chemistry*. *35*(7). 1843-1851.

Khangarot. B. S.. & Ray. P. K., 1988. Sensitivity of freshwater pulmonate snails. Lymnaea luteolo L.. to heavy metals. *Bull. Environ. Contam. Toxicol.;(United States)*. *41*(2).

Sánchez-Moreno. S.. Camargo. J. A.. & Navas. A., 2006. Ecotoxicological assessment of the impact of residual heavy metals on soil nematodes in the Guadiamar River Basin (Southern Spain). *Environmental Monitoring and Assessment*. *116*. 245-262.

Khangarot. B. S.. Mathur. S.. & Durve. V. S., 1982. Comparative toxicity of heavy metals and interaction of metals on a freshwater pulmonate snail Lymnaea acuminata (Lamarck). *Acta hydrochimica et hydrobiologica*. *10*(4). 367-375.

Pickering. Q. H.. & Henderson. C., 1966. The acute toxicity of some heavy metals to different species of warmwater fishes. *Air and water pollution*. *10*(6). 453-463.

Gupta. P. K.. Durve. V. S.. & Khangarot. B. S., 1981. Studies on the acute toxicity of some heavy metals to an Indian freshwater pond snail Viviparus bengalensis L. *Arch. Hydrobiol.;(Germany. Federal Republic of)*. *91*(2).

Shuhaimi-Othman. M.. Yakub. N.. Ramle. N. A.. & Abas. A. (2011). Toxicity of metals to a freshwater ostracod: Stenocypris major. *Journal of Toxicology*. *2011*.

Powlesland. C.. & George. J., 1986. Acute and chronic toxicity of nickel to larvae of Chironomus riparis (Meigen). *Environmental Pollution Series A. Ecological and Biological*. *42*(1). 47-64.

Haight. M.. Mudry. T.. & Pasternak. J., 1982. Toxicity of Seven Heavy Metals On Panagrell Us Silusiae: the Efficacy of the Free-Living Nematode as an in Vivo Toxicological Bioassay. *Nematologica*. *28*(1). 3-11.

Sharma. M. S.. & Selvaraj. C. S., 1994. Zinc. lead and cadmium toxicity to selected freshwater zooplankters. *Pollution Research*. *13*(2). 191-201.

Shuhaimi-Othman. M.. Yakub. N.. Umirah. N. S.. & Abas. A. (2011). Toxicity of eight metals to Malaysian freshwater midge larvae Chironomus javanus (Diptera. Chironomidae). *Toxicology and industrial health*. *27*(10). 879-886.

Ziegenfuss. P. S.. Renaudette. W. J.. & Adams. W. J., 1986. Methodology for assessing the acute toxicity of chemicals sorbed to sediments: testing the equilibrium partitioning theory. *Aquatic toxicology and environmental fate*. *9*. 479-493.

Ha. M. H.. & Choi. J., 2008. Effects of environmental contaminants on hemoglobin of larvae of aquatic midge. Chironomus riparius (Diptera: Chironomidae): A potential biomarker for ecotoxicity monitoring. *Chemosphere*. *71*(10). 1928-1936.

Cairns Jr. J.. Messenger. D. I.. & Calhoun. W. F., 1976. Invertebrate response to thermal shock following exposure to acutely sub-lethal concentrations of chemicals. *Arch. Hydrobiol.*. *77*(2). 164-175.

Bat. L.. Gündoğdu. A.. Akbulut. M.. Çulha. M.. & Satılmış. H. H., 2001. Toxicity of zinc and lead to the polychaete (Hediste diversicolor Müller 1776).

Woodiwiss. F. S.. & Fretwell. G., 1974. The toxicities of sewage effluents. industrial discharges and some chemical substances to brown trout (Salmo trutta) in the Trent River Authority area. *Water Pollution Control (UK)*.

Boutet. C.. & Chaisemartin. C., 1973. Specific toxic properties of metal salts of Austropotamobius pallipes pallipes and Orconectes limosus. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales*. *167*(12). 1933-1938.

Vincent. M.. Debord. J.. & Penicaut. B., 1986. Comparative study of the toxicity of metal chlorides and a synthetic organic molluscacide. N-tritylmorpholine. in 2 fresh-water amphipods. Gammarus pulex and Echinogammarus berilloni. *Annales de Recherches veterinaires. Annals of Veterinary Research*. *17*(4). 441-446.

Tooby. T. E.. Hursey. P. A.. & Alabaster. J. S., 1975. The acute toxicity of 102 pesticides and miscellaneous substances to fish. *Chem. Ind*. *12*. 523.

LeBlanc. G. A., 1980. Acute toxicity of priority pollutants to water flea (Daphnia magna). *Bull. Environ. Contam. Toxicol*. *24*(5). 684-691.

U.S. Environmental Protection Agency. In-Depth Studies on Health and Environmental Impacts of Selected Water Pollutants. U.S.EPA Contract No.68-01-4646. Duluth. MN:9 p.. 1978

Calleja. M. C.. Persoone. G.. & Geladi. P., 1994. Comparative acute toxicity of the first 50 multicentre evaluation of in vitro cytotoxicity chemicals to aquatic non-vertebrates. *Archives of Environmental Contamination and Toxicology*. *26*. 69-78.

Juhnke. I.. & Luedemann. D., 1978. Results of the investigation of 200 chemical compounds for acute fish toxicity with the golden orfe test. *Journal of Water and Wastewater Research*. *11*. 161-164.

Dorfman. D., 1977. Tolerance of Fundulus heteroclitus to different metals in salt waters. *Bull NJ Acad Sci*. *22*(2). 21-23.

Wallen. I. E.. Greer. W. C.. & Lasater. R., 1957. Toxicity to" Gambusia Affinis" of Certain Pure Chemicals in Turbid Waters. *Sewage and Industrial Wastes*. *29*(6). 695-711.

Hu. J.. Wang. D.. Forthaus. B. E.. & Wang. J., 2012. Quantifying the effect of nanoparticles on As (V) ecotoxicity exemplified by nano-Fe2O3 (magnetic) and nano-Al2O3. *Environmental Toxicology and Chemistry*. *31*(12). 2870-2876.

Abdelghani. A. A.. Anderson. A. C.. & McDonell. D. B., 1980. Toxicity of three arsenical compounds. *Can Res*. *13*. 31-32.

Tišler. T.. & Zagorc-Končan. J., 2002. Acute and chronic toxicity of arsenic to some aquatic organisms. *Bulletin of environmental contamination and toxicology*. *69*. 421-429.

Hashimoto.Y.. and Y. Nishiuchi., 1981. Establishment of bioassay methods for the evaluation of acute toxicity of pesticides to aquatic organisms. *Journal of Pesticide Science*. *6*(2). 257-264.

Schaefer. E. D.. & Pipes. W. O., 1973. Temperature and the toxicity of chromate and arsenate to the rotifer. Philodina roseola. *Water Research*. *7*(12). 1781-1790.

Jeyasingham. K.. & Ling. N., 2000. Acute toxicity of arsenic to three species of New Zealand chironomids: Chironomus zealandicus. Chironomus sp. a and Polypedilum pavidus (Diptera. Chironomidae). *Bulletin of environmental contamination and toxicology*. *64*(5). 708-715.

Holcombe. G. W.. Phipps. G. L.. & Fiandt. J. T., 1983. Toxicity of selected priority pollutants to various aquatic organisms. *Ecotoxicology and Environmental Safety*. *7*(4). 400-409.

Curtis. M. W.. Copeland. T. L.. & Ward. C. H., 1979. Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. *Water Research*. *13*(2). 137-141.

Rao. R. S. V.. & Nath. K. J., 1983. Biological effect of some poisons on Canthocamptus (Crustacea spp). *International journal of environmental studies*. *21*(3-4). 271-275.

Fargašová. A., 1999. Ecotoxicology of metals related to freshwater benthos. *General Physiology and Biophysics*. 48-53.

Shuhaimi-Othman. M.. Nadzifah. Y.. Umirah. N. S.. & Ahmad. A. K., 2012. Naididae. *Research Journal of Environmental Toxicology*. *6*(4). 122-132.

Rao. I. J.. & Madhyastha. M. N., 1987. Toxicities of some heavy metals to the tadpoles of frog. Microhyla ornata (Dumeril & Bibron). *Toxicology Letters*. *36*(2). 205-208.

Slabbert. J. L.. & Venter. E. A., 1999. Biological assays for aquatic toxicity testing. *Water science and technology*. *39*(10-11). 367-373.

Hockett. J. R.. & Mount. D. R., 1996. Use of metal chelating agents to differentiate among sources of acute aquatic toxicity. *Environmental Toxicology and Chemistry: An International Journal*. *15*(10). 1687-1693.

Oyewo. E. O.. & Don-Pedro. K. N., 2006. Acute toxicity and induced weight changes in laboratory tests with Mn and Cu against Tilapia guineensis(Dumeril) and Tympanotonus fuscatus(Linne). *Journal of Environmental Biology*. *37*(2). 327-334.

Nath. K.. & Kumar. N., 1987. Toxicity of manganese and its impact on some aspects of carbohydrate metabolism of a freshwater teleost. Colisa fasciatus. *Science of the Total Environment*. *67*(2-3). 257-262.

Bu-Olayan. A. H.. & Thomas. B. V., 2008. Trace metals toxicity and bioaccumulation in mudskipper Periophthalmus waltoni Koumans 1941 (Gobiidae: Perciformes). Turkish Journal of Fisheries and Aquatic Sciences. 8(2).

Alam. M. K.. & Maughan. O. E., 1995. Acute toxicity of heavy metals to common carp (Cyprinus carpio). Journal of Environmental Science & Health Part A. 30(8). 1807-1816.

Birge. W. J.. Black. J. A.. Westerman. A. G.. Short. T. M.. Taylor. S. B.. Bruser. D. M.. & Wallingford. E. D., 1985. Recommendations on numerical values for regulating iron and chloride concentrations for the purpose of protecting warm water species of aquatic life in the Commonwealth of Kentucky. Memorandum of Agreement. 5429.

Dowden. B. F.. & Bennett. H. J., 1965. Toxicity of selected chemicals to certain animals. Journal (Water Pollution Control Federation). 1308-1316.

Fort. D. J.. & Stover. E. L., 1995. Impact of toxicities and potential interactions of flocculants and coagulant aids on whole effluent toxicity testing. Water environment research. 67(6). 921-925.

Khan. S.. & Nugegoda. D., 2007. Sensitivity of juvenile freshwater crayfish Cherax destructor (Decapoda: Parastacidae) to trace metals. Ecotoxicology and environmental safety. 68(3). 463-469.

Furmanska. M., 1979. Studies of the effect of copper. zinc and iron on the biotic components of aquatic ecosystems.

Supplementary Material – Chapter 3

Toxicity of isolated and mixed metals to a native Amazonian ostracod and ecological risk assessment

Table S1 - Nominal concentrations used in ecotoxicological tests (mg L^{-1}) of metals: Copper Sulfate ($CuSO_4$), Cadmium Chloride ($CdCl_2$), Mercury Chloride ($HgCl_2$) e Zinc Chloride ($ZnCl_2$).

Metal	**Concentrations (mg L^{-1})**
$CuSO_4$	0.50; 1.0; 1.50; 2.0; 4.0;
$CdCl_2$	0.10; 0.20; 0.40; 0.60; 0.80;
$ZnCl_2$	10; 20; 40; 60; 80;
$HgCl_2$	0.10; 0.20; 0.40; 0.80; 1.60;

Table S2 – Values of nominal concentrations, measurements and metal detection limits for the stock solutions used in toxicity tests in this study.

Stock solution concentration	Metal	Nominal Concentration (mg L^{-1})	Quantified Concentration (mg L^{-1})	Detection limit	Reference Method
1000 mg L^{-1}	Cd	610	661.3 ± 0.57	0.0006	SMWW 3111 B
	Cu	254.3	265 ± 1.5	0.003	SMWW 3111 B
	Zn	470.9	507.3 ± 2.1	0.002	SMWW 3111 B
	Hg	730.8	690.3 ± 27.3	2,000000E-6	USEPA 1631 E / USEPA 1630
10 mg L-1	Cd	6.1	6.69 ± 0.05	0.0006	SMWW 3111 B
	Cu	2.45	2.31 ± 0.08	0.003	SMWW 3111 B
	Zn	4.7	5.21 ± 0.01	0.002	SMWW 3111 B
	Hg	7.3	7.29 ± 0.2	2,000000E-6	USEPA 1631 E / USEPA 1630

Table S3 – Values of lethal concentrations and references used for the construction of SSD curves.

Metal	LC50	Species	Group	Authors
CuSO4	0.0134	*Ceriodaphnia dubia*	Crustacean	Oris, Winner, Moore (1991)
	0.02575	*Allonais inaequalis*	Annelid	Rocha et al. (2018)
	0.03139	*Dero furcatus*	Annelid	Rocha et al. (2018)
	0.038	*Stenocypris major*	Crustacean	Shuhaimi-Othman et al. (2011)
	0.055	*Cypris subglobosa*	Crustacean	Khangarot, Ray (1987)
	0.056	*Daphnia magna*	Crustacean	Debelak and Robert (1975)
	0.1	*Chironomus tentans*	Insect	Ziegenfuss, Renaudette, Adams, (1986)
	0.107	*Daphnia laevis*	Crustacean	Rocha et al. (2016)
	0.15	*Pimephales promelas*	Fish	Curtis, Copeland, Ward, (1978)
	0.21	*Tubifex tubifex*	Annelid	Brković-Popović, Popović, (1977)
	0.23	*Lumbriculus variegatus*	Annelid	Bailey and Liu (1980)
	0.59	*Limnodrilus hoffmeisteri*	Annelid	Wurtz, Bridges (1961)
	0.611	*Tramea sp.*	Insect	dos Santos et al. (2019)
	0.749	*Strandesia trispinosa*	Crustacean	dos Santos et al. (2019)
	0.78	*Diacypris compacta*	Crustacean	Brooks, White, Paton (1995)
	0.82	*Lymnaea sp.*	Clam	Sullivan, Palmieri, Agoes (1977)
	1.4	*Cephalobus persegnis*	Nematode	Sanchez-Moreno, Camargo, Navas (2006)
	1.7	***Strandesia rondoniensis***	**Crustacean**	**This study**
	2.09	*Chironomus riparius*	Insect	Béchard, Gillis, Wood, (2008)
	2.6	*Cypridopsis Vidua*	Crustacean	Chen et al. (2022)
	5.79	*Heterocypris incongruens*	Crustacean	Janssen and persoone (2011)
ZnCl²	0.183	*Ceriodaphnia dubia*	Crustacean	Carlson, Nelson, Hammermeister (1986)
	0.79	*Daphnia magna*	Crustacean	Attar, E. N., & Maly, E. J. (1982)
	1.68	*Stenocypris major*	Crustacean	Shuhaimi-Othman et al. (2011)
	1.82	*Neanthes arenaceodentata*	Annelid	Reish (1977)
	2.45	*Oreochromis mossambicus*	Fish	Qureshi, Saksena, (1980)
	2.85	*Ranatra elongata*	Insect	Shukla, Murti (1983)
	6.76	*Poecilia reticulata*	Fish	Anderson, Weber (1975)
	7.24	*Lepomis macrochirus*	Fish	Pickering, Henderson (1966)
	8.1	*Lumbriculus variegatus*	Annelid	Bailey, Liu (1980)
	9.56	*Lymnaea stagnalis*	Molluscs	Vyskushenko (2006)
	48.94	***Strandesia rondoniensis***	**Crustacean**	**This study**
	52	*Austropotamobius pallipes*	Crustacean	Boutet, Chaisemartin, (1973)
	58	*Orconectes limosus*	Crustacean	Boutet, Chaisemartin, (1973)
	80	*Chironomus sp.*	Insect	Qureshi, Saksena, Singh, (1980)
	85.04	*Cypris subglobosa*	Crustacean	Khangarot, Das, (2009)
	94.3	*Chironomus riparius*	Insect	Ibrahim et al. (1998)
	130	*Tubifex tubifex*	Annelid	Qureshi, Saksena, Singh (1980)
CdCl²	0.012	*Strandesia trispinosa*	Crustacean	dos Santos et al. (2019)
	0.022	*Stenocypris major*	Crustacean	Shuhaimi-Othman et al. (2011)
	0.03	*Daphnia magna*	Crustacean	Canton, J. H., & Slooff, W. (1982).

	0.063	*Ceriodaphnia dubia*	Crustacean	Suedel, Rodgers, Deaver (1997)
	0.082	*Hydra viridissima*	Cndarium	Holdway, Lok, Semaan, (2001)
	0.12	*Lumbriculus variegatus*	Annelid	Bailey and Liu (1980)
	0.19	*Cypridopsis sp.*	Crustacean	Fennikoh, Hirshfield, Kneip (1978)
	0.29	*Chironomus tentans*	Insect	Hooftman et al. (1989)
	0.29	*Macrobrachium lamarrei*	Crustacean	Murti and Shukla (1984)
	0.34	*Macromia sp.*	Insect	Fennikoh, Hirshfield, Kneip (1978)
	0.364	*Deru furcatus*	Annelid	Rocha et al. (2018)
	0.511	***Strandesia rondoniensis***	**Crustacean**	**This study**
	0.627	*Allonais inaequalis*	Annelid	Rocha et al. (2018)
	0.7	*Tubifex tubifex*	Annelid	Brkovic-Popovic, Popovic, (1977)
	0.821	*Cypris subglobosa*	Crustacean	Khangarot and Das, (2009)
	0.844	*Tramea sp.*	Insect	dos Santos et al. (2019)
	11.85	*Melanoides tuberculata*	Clam	Shuhaimi-Othman, et al. (2012)
	20.5	*Poecilia reticulata*	Fish	Yılmaz, Gül, Karaköse (2004)
	36.58	*Branchiura sowerbyi*	Annelid	Das, Kaviraj (1994)
$HgCl^2$	0.0003	*Daphnia magna*	Crustacean	Canton, Adema (1978).
	0.008	*Ceriodaphnia dubia*	Crustacean	Valenti et al. (2007)
	0.02739	*Strandesia trispinosa*	Crustacean	dos Santos et al. (2019)
	0.039	*Tubifex tubifex*	Annelid	Rathore, Khangarot (2002)
	0.0635	*Daphnia similis*	Crustacean	Soundrapandian, Venkataraman (1990)
	0.092	*Deru Furcatus*	Annelid	Rocha et al. (2018)
	0.097	*Cypris subglobosa*	Crustacean	Khangarot and Das, (2009)
	0.129	*Allonais inaequalis*	Annelid	Rocha et al. (2018)
	0.13	*Cypris sp.*	Crustacean	Qureshi, Saksena, Singh (1980)
	0.18	*Limnodrilus hoffmeisteri*	Annelid	Chapman, Farrell, Brinkhurst (1982)
	0.316	*Chironomus riparius*	Insect	Rossaro, Gaggino, Marchetti (1986)
	0.7595	***Strandesia rondoniensis***	**Crustacean**	**This study**
	0.84553	*Tramea sp.*	Insect	dos Santos et al. (2019)
	1.05	*Culex pipiens*	Insect	Slooff, Canton, Hermens, (1983)
	1.8	*Chironomus sp.*	Insect	Qureshi, Saksena, Singh (1980)
	3.75	*Chironomus tentans*	Insect	Ziegenfuss, Renaudette, Adams (1986)
	5.6	*Aedes aegypti*	Insect	Slooff (1982)
	7.613	*Ischnura elegans*	Insect	Slooff (1983)
	42.2	*Chironomus plumosus*	Insect	Vedamanikam, Shazili (2009)

Table S4 - HC_5 e HC_{50} (danger concentration for 5% and 50% of species, respectively; in mg L^{-1}) and 95% confidence intervals for copper (Cu), cadmium (Cd), mercury (Hg) e zinc (Zn), from the curves DSEs built based on the values of LC_{50} for different groups of aquatic organisms (Figure 6).

Metal	HC_5		HC_{50}	
	HC5	CI 95%	HC50	CI 95%
Cu	0.0151	0.004 - 0.035	0.271	0.141 - 0.520
Zn	0.3847	0.078 - 1.09	10.21	4.46 - 23.39
Cd	0.010	0.002 - 0.030	0.384	0.162 - 0.910
Hg	0.005	0.0008 - 0.017	0.299	0.113 - 0.791

Table S5 – Average values and standard deviations of physical and chemical variables: pH, Dissolved Oxygen - O.D - (mg L^{-1}), Electric conductivity – C.E – (µs/cm) and temperature – T – (C°), Hardness - ($CaCO_3$); measurements at the beginning and end of toxicity experiments with isolated metals and in mixtures with ostracod *Strandesia rondoniensis*.

Metals	Variables	Initial	Final
Copper Sulfate ($CuSO_4$)	pH	7.48 ± 0.17	7.52 ± 0.10
	O.D	6.44 ± 0,59	6.28 ± 0.62
	C.E	154.3 ± 7.55	159.7 ± 15.26
	T	24.86 ± 0.68	25.3 ± 0.49
	Hardness	44 ± 2.8	-
Zinc Chloride ($ZnCl_2$)	pH	7.53 ± 0.08	7,59 ± 0,12
	O.D	6.14 ± 0.72	5.85 ± 0.60
	C.E	139.9 ± 4.19	142.93 ± 4.12
	T	24.79 ± 0.61	25.00 ± 0.72
	Hardness	44 ± 3.65	-
Cadmium Chloride ($CdCl_2$)	pH	7.64 ± 0.08	7.73 – 0.10
	O.D	6.73 ± 0.03	6.63 ± 0.13
	C.E	138.83 ± 11.85	146.03 ± 17.12
	T	25.17 ± 0.38	25.25 ± 0.42
	Hardness	44 ± 2.82	-
Mercury Chloride ($HgCl_2$)	pH	7.57 ± 0.13	7.65 ± 0.26
	O.D	6.06 ± 0.83	5.37 ± 0.49
	C.E	144.36 ± 19.84	147.86 ± 17.95
	T	25.27 ± 0.64	24.87 ± 0.58
	Hardness	42 ± 1.6	-
Copper Sulfate ($CuSO_4$) X Zinc Chloride ($ZnCl_2$)	pH	7.77 ± 0,1	7.83 ± 0,3
	O.D	6.8 ± 0.5	5.8 ± 0.5
	C.E	153.20 ± 13	160.41 ± 19.2
	T	24.9 ± 0.48	25.2 ± 0.25
	Hardness	42 ± 0.8	-
Copper Sulfate ($CuSO_4$) X Cadmium Chloride ($CdCl_2$)	pH	7.65 ± 0.52	7.89 ± 0.39
	O.D	7.29 ± 0.82	6.47 ± 0.39
	C.E	139.2 ± 17	167 ± 12
	T	25.33 ± 1.75	25.62 ± 1.33
	Hardness	44 ± 0.6	-
Copper Sulfate ($CuSO_4$) X Mercury Chloride ($HgCl_2$)	pH	7.34 ± 0.74	7.88 ± 0.43
	O.D	6.86 ± 0.60	5.87 ± 0.32
	C.E	159 ± 10	167 ± 8
	T	24.9 ± 0.38	25.5 ± 0.47
	Hardness	42 ± 0.4	-

Table S6 – Average values of $LC_{50/48/72/96H}$ and respective confidence intervals for 95% (mg L^{-1}) for metals copper sulfate ($CuSO_4$), cadmium chloride ($CdCl_2$), mercury chloride ($HgCl_2$) and zinc chloride ($ZnCl_2$) obtained in acute toxicity tests for ostracod *Strandesia rondoniensis.*

Test Organism	Metal	$LC_{50/48H}$	$LC_{50/72H}$	$LC_{50/96H}$
Strandesia rondoniensis	$CuSO_4$	1.70 (1.44 – 1.92)	1.05 (0.88 – 1.23)	0.67 (0.55 – 0.79)
	$CdCl_2$	0.37 (0.27 – 0.47)	0.08 (0.05 – 0.10)	0.04 (0.0009 – 0.08)
	$ZnCl_2$	54.87 (48.8 – 60.85)	27.16 (22.1 – 32.1)	13.13 (10.78 – 15.47)
	$HgCl_2$	0.95 (0.82 – 1.07)	0.45 (0.32 – 0.59)	0.14 (0.09 - 0.19)

Table S7 – Values of $LC_{50/48H}$ for metals Cu, Cd, Hg, Zn and Water hardness ($CaCO_3$), selected to compare sensitivity between different species of ostracods, (*) data in $EC_{50/48H}$.

Species	Cu	Cd	Zn	Hg	Hardness	authors
Strandesia rondoniensis	1.69	0.37	54.87	0.95	40-44	**This study**
*Cypris subglobosa**	0.55	0.821	85.04	0.369	245	Khangarot; Das, (2009)
Heterocypris incongruens	5.79	0.053	-	-	160-180	Janssen; P. (2015); Gutiérrez, (2010)
Strandesia trispnosa	0.75	0.012	-	0.027	188	Lima et al., (2020)
Chlamydotheca sp.	0.378	0.073	-	0.749	188	Lima et al., (2020)
Stenocypris major	0.038	0.05073	1.682	-	15.63	Shuhaimi-Othman, (2011)
Heterocypris bosniaca	-	0.61	-	-	160-180	Aguilar-A.; Mesquita-Joanes (2012)
Chlamydotheca incisa	-	0.307	-	-	215	Liberto, (2010)
Strandesia bicuspis	-	0.039	-	-	215	Liberto, (2010)

Table S8 - Results of the analysis of the acute toxicity test of mixtures of $CuSO_4$ and $CdCl_2$. For adult individuals of the species *Strandesia rondoniensis.*

	CA	S/A	DR	DL	AI	S/A	DR	DL
Max	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
βCu	6.645478	5.797794	5.18164	4.910247	2.851967	3.741222	4.201315	4.759757
βCd	2.628618	2.462518	2.779077	2.237033	2.316612	3.156203	3.071445	3.847335
CE50 Cu	1.555851	1.714859	1.735008	1.663961	1.043732	1.688077	1.809174	1.650437
CE50 Cd	0.7686	0.988251	0.964952	0.952825	0.681941	0.984538	0.951556	0.932467
A	-	-1.30913	-0.26392	0.004622	-	-5.97445	-2.60803	-7.80389
bDR/DL	-	-	-1.91962	234.6204	-	-	-7.29676	0.610816
SS	59.82904	47.83075	46.51643	52.05579	101.2122	55.70569	50.66018	53.39031
R2	0.84	0.876955	0.880336	0.866086	0.73963	0.856696	0.869676	0.862653
χ2 ou teste F	328.8955	11.99829			287.5124			
Df	-	1	1	1		1	2	2
p (χ2/ F)	6.31E-70	0.000532	0.001286	0.080514	5.35E-61	1.52E-11	0.02469	0.1281

max = maximum value of the response; β = slope of the individual dose response curve; EC50 = median effective concentration; The. bDR and bDL = function parameters; SS = sum of squares of residuals; r^2 = regression coefficient;

χ2 or F test = statistical test; df = degree of freedom; p (χ2 / F) = significance level for the test statistic. IA = independent action model. AC = is the concentration addition model. S/A = deviation synergism/antagonism. DR = dose ratio dependent deviation and DL = dose level dependent deviation.

Table S9 - Results of the analysis of the acute toxicity test of mixtures of $CuSO_4$ e $ZnCl_2$. For adult individuals of the species *Strandesia rondoniensis.*

	CA	S/A	DR	DL	AI	S/A	DR	DL
Max	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
βCu	4.0326329	4.4747471	4.4003732	5.8109982	4.1523095	4.0596895	3.9976959	4.5801551
βCd	2.7838582	4.6399911	4.7982249	5.5516494	4.0529783	3.9834759	4.0904318	4.5935035
CE50 Cu	2.1325138	1.6060887	1.6572589	1.7652362	1.7457385	1.6330228	1.6839462	1.6049835
CE50 Cd	71.060481	50.862544	49.554956	55.745445	54.926841	51.186705	49.83909	49.921706
A	-	2.172906	2.6946108	-0.00689	-	1.1115321	2.4350227	0.0051927
bDR/DL	-	-	-1.076501	147.53119	-	-	-2.6999624	-461.69352
SS	74.161005	32.533014	31.838402	45.537899	38.201598	36.514734	35.929988	34.478195
R2	0.7975239	0.9111776	0.913074	0.8756714	0.8957011	0.9003066	0.9019031	0.9058668
χ2 ou teste F	292.10939	-	-	-	328.0688	-	-	-
Df	-	1	2	1	-	1	2	1
p (χ2/ F)	5.454E-62	1.104E-10	6.453E-10	-	9.513E-70	0.1940149	0.444458	0.1535587

max = maximum value of the response; β = slope of the individual dose response curve; EC50 = median effective concentration; The. bDR and bDL = function parameters; SS = sum of squares of residuals; r^2 = regression coefficient; χ2 or F test = statistical test; df = degree of freedom; p (χ2 / F) = significance level for the test statistic. IA = independent action model. AC = is the concentration addition model. S/A = deviation synergism/antagonism. DR = dose ratio dependent deviation and DL = dose level dependent deviation.

Table S10 - Results of the analysis of the acute toxicity test of mixtures from $CuSO_4$ and $HgCl_2$. For adult individuals of the species *Strandesia rondoniensis.*

	CA	S/A	DR	DL	AI	S/A	DR	DL
Max	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
βCu	7.119509	7.264125	6.165818	6.398572	4.607281	4.884064	5.13950933	4.420347
βCd	1.821893	1.453333	2.00808	0.918256	1.307061	1.503303	2.27324391	1.129526
CE50 Cu	1.299363	1.453677	1.452649	1.461729	1.050294	1.26547	1.38009544	1.291298
CE50 Cd	0.309587	0.398	0.3849	0.384204	0.228168	0.3341	0.36148777	0.317981
A	-	-1.67308	1.777694	0.044989	-	-3.49304	3.66704287	-0.02226
bDR/DL	-	-	-5.77998	42.46058	-	-	-17.336189	-289.193
SS	62.77204	49.90758	43.98458	46.9053	74.28314	56.53987	39.1664459	52.08247
R2	0.824421	0.860404	0.876971	0.868802	0.792223	0.841853	0.89044798	0.854321
χ2 ou teste F	294.7425	-	-	-	283.2314	-	-	-
Df	-	1	2	1	-	1	2	1
p (χ2/ F)	1.48E-62	0.000335	8.32E-05	0.000359	4.48E-60	2.53E-05	3.0709E-05	1.51E-05

max = maximum value of the response; β = slope of the individual dose response curve; EC50 = median effective concentration; The. bDR and bDL = function parameters; SS = sum of squares of residuals; r^2 = regression coefficient; $\chi 2$ or F test = statistical test; df = degree of freedom; p ($\chi 2$ / F) = significance level for the test statistic. IA = independent action model. AC = is the concentration addition model. S/A = deviation synergism/antagonism. DR = dose ratio dependent deviation and DL = dose level dependent deviation.

Tabela S11 – Metal concentrations (mg L^{-1}) used to conduct the Ecological Risk Assessment.

Cu			**Zn**			**Hg**			**Cd**		
PEC Min	PEC Med	PEC Max	PEC Min	PEC Med	PEC Max	PEC Min	PEC Med	PEC Max	PEC Min	PEC Med	PEC Max
0.071	8.053	82.119	0.0052	0.16	0.7240	0.0160	0.0280	20.00	1.20	864.0	3064.0
Santarém - Pará - Morgado, (2019)	All Locations - Gomes et al (2023)	Manaus - Am - da Silva, (2010)	Mago Grosso - MT - Eidt, (2015)	All Locations - Gomes et al (2023)	Amapá - AP - Lima et al. (2015)	All Locations - Moulatlet et al. (2023)	All Locations - Moulatlet et al. (2023)	All Locations - Moulatlet et al. (2023)	Tocantins - TO - Duarte (2013)	All Locations - Gomes et al (2023)	Manaus - AM - Silva, (2010)

References SSD

Adams. W. J., Heidolph. B. B. (1985). Short-cut chronic toxicity estimates using Daphnia magna. ASTM International.

Ahsanullah. M. (1976). Acute toxicity of cadmium and zinc to seven invertebrate species from Western Port. Victoria. Marine and Freshwater Research. 27(2). 187-196.

Anderson. P. D., & Weber. L. J. (1975). Toxic response as a quantitative function of body size. Toxicology and applied pharmacology. 33(3). 471-483.

Arnott. G. H., & Ahsanullah. M. (1979). Acute toxicity of copper. cadmium and zinc to three species of marine copepod [heavy metal pollution; zooplankton]. Australian Journal of Marine and Freshwater Research (Australia).

Bailey. H. C., & Liu. D. H. W. (1980). Lumbriculus variegatus. a benthic oligochaete. as a bioassay organism (pp. 202-215). ASTM International.

Boutet. C., & Chaisemartin. C. (1973). Specific toxic properties of metal salts of Austropotamobius pallipes pallipes and Orconectes limosus. Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales. 167(12). 1933-1938.

Brooks. A., White. R. M., Paton. D. C. (1995). Effects of heavy metals on the survival of *Diacypris compacta* (Herbst) (Ostracoda) from the Coorong. South Australia. *International Journal of Salt Lake Research*. 4. 133-163.

Burton. D. T., & Fisher. D. J. (1990). Acute toxicity of cadmium. copper. zinc. ammonia. 3. 3 prime-dichlorobenzidine. 2. 6-dichloro-4-nitroaniline. methylene chloride. and 2. 4. 6-trichlorophenol to juvenile grass shrimp and killifish. Bulletin of Environmental Contamination and Toxicology;(USA). 44(5).

Carlson. A. R., Nelson. H., & Hammermeister. D. E. (1986). Evaluation of site-specific criteria for copper and zinc: an integration of metal addition toxicity. effluent and receiving water toxicity. and ecological survey data (No. PB-86-183928/XAB; EPA/600/3-86/026). Environmental Protection Agency. Duluth. MN (USA). Environmental Research Lab.,

Chapman. P. M., Farrell. M. A., & Brinkhurst. R. O. (1982). Relative tolerances of selected aquatic oligochaetes to individual pollutants and environmental factors. Aquatic toxicology. 2(1). 47-67.

Cowgill. U. M., Milazzo. D. P. (1991). Comparison of the effect of metallic copper and copper nitrate (Cu (No 3) 2· 3H 2 O) on Ceriodaphnia dubia utilizing the three-brood test. *Bulletin of environmental contamination and toxicology*. 46. 141-145.

Das. B. K., Kaviraj. A. (1994). Individual and interactive lethal toxicity of cadmium. potassium permanganate and cobalt chloride to fish. worm and plankton. *GEOBIOS-JODHPUR-*. 21. 223-223.

dos Santos Lima. J. C., Neto. A. J. G., de Pádua Andrade. D., Freitas. E. C., Moreira. R. A., Miguel. M., Rocha. O. (2019). 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*. 180. 535-541.

Espiritu. E. Q., Teran. A. B., Antonio. R. L. (2011). Development of Standard Toxicity Tests for Tropical Aquatic Environments: 1. The Use of Tilapia. *Oreochromis niloticus* (L.). as Toxicity Test Organism for Freshwater Habitats. Philippine Scientist. 48.

Fan. W., Shi. Z., Yang. X., Cui. M., Wang. X., Zhang. D., Guo. L. (2012). Bioaccumulation and biomarker responses of cubic and octahedral Cu2O micro/nanocrystals in Daphnia magna. *Water research*. 46(18). 5981-5988.

Fennikoh. K. B., Hirshfield. H. I., Kneip. T. J. (1978). Cadmium toxicity in planktonic organisms of a freshwater food web. *Environmental research*. 15(3). 357-367.

Govindarajan. S., Valsaraj. C. P., Mohan. R., Hariprasad. V., Ramasubramanian. R. (1993). Toxicity of heavy metals in aquaculture organisms: *Penaeus indicus*. *Perna viridis*. Artemia salina and Skeletonema costatum. *Pollut. Res*. 12(3). 187-189.

Guth. D. J., Blankespoor. H. D., & Cairns. J. (1977). Potentiation of zinc stress caused by parasitic infection of snails. Hydrobiologia. 55. 225-229.

Holdway. D. A., Lok. K., Semaan. M. (2001). The acute and chronic toxicity of cadmium and zinc to two hydra species. *Environmental toxicology*. 16(6). 557-565.

Hooftman. R. N., Adema. D. M. M., Kauffman-Van Bommel. J. (1989). Developing a set of test methods for the toxicological analysis of the pollution degree of waterbottoms.

Ibrahim. H., Kheir. R., Helmi. S., Lewis. J., & Crane. M. (1998). Effects of organophosphorus. carbamate. pyrethroid and organochlorine pesticides. and a heavy metal on survival and cholinesterase activity of Chironomus riparius Meigen. Bulletin of Environmental Contamination and Toxicology. 60. 448-455.

Janssen C.R. and Persoone G., (2011). International interlaboratory comparison on the subchronic toxicity test with the freshwater ostracod crustacean *Heterocypris incongruens*. Report in www. microbiotests.be. section "Publications and Reports". 1–16.

Khangarot. B. S., & Das. S. (2009). Acute toxicity of metals and reference toxicants to a freshwater ostracod. Cypris subglobosa Sowerby. 1840 and correlation to EC50 values of other test models. *Journal of hazardous materials*. 172(2-3). 641-649.

Khangarot. B. S., Ray. P. K. (1987). Response of a freshwater ostracod (*Cypris subglobosa* Sowerby) exposed to copper at different pH levels. *Acta hydrochimica* et hydrobiologica. 15(6). 553-558.

Koryakova. M. (1993). Using barnacle larvae for evaluation of the toxicity of antifouling paint compounds. *Russ J Mar Biol*. 19. 212-216.

Lee. S. I., Na. E. J., Cho. Y. O., Koopman. B., Bitton. G. (1997). Short-term toxicity test based on algal uptake by Ceriodaphnia dubia. *Water environment research*. 69(7). 1207-1210.

Meyer. J. S., Boese. C. J., Collyard. S. A. (2002). Whole-body accumulation of copper predicts acute toxicity to an aquatic oligochaete (*Lumbriculus variegatus*) as pH and calcium are varied. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 133(1-2). 99-109.

Murti. R., Shukla. G. S. (1984). Acute toxicity of mercuric chloride and cadmium chloride to freshwater prawn. Macrobrachium lamarrei (H. Milne Edwards). *Acta hydrochimica et hydrobiologica*. 12(6). 689-692.

Nebeker. A. V., Cairns. M. A., Wise. C. M. (1984). Relative sensitivity of *Chironomus tentans* life stages to copper. *Environmental Toxicology and Chemistry*: An International Journal. 3(1). 151-158.

Pickering. Q. H., Henderson. C. (1966). The acute toxicity of some heavy metals to different species of warmwater fishes. Air and water pollution. 10(6). 453-463.

Qureshi. S. A., Saksena. A. B. (1980). The acute toxicity of some heavy metals to Tilapia mossambica (Peters). Aquat Sci Tech Rev. 1. 19-20.

Qureshi. S. A., Saksena. A. B., Singh. V. P. (1980). Acute toxicity of four heavy metals to benthic fish food organisms from the River Khan. Ujjain.

Rathore. R. S., Khangarot. B. S. (2002). Effects of temperature on the sensitivity of sludge worm Tubifex tubifex Müller to selected heavy metals. *Ecotoxicology and environmental safety*. 53(1). 27-36.

Reish. D. J. (1977). Toxicity of Formulated Mine Tailings on Marine Polychaeta. Fisheries and Marine Service.

Reynoldson. T. B., Rodriguez. P., & Madrid. M. M. (1996). A comparison of reproduction. growth and acute toxicity in two populations of Tubifex tubifex (Müller.

1774) from the North American Great Lakes and Northern Spain. In Aquatic Oligochaete Biology VI: Proceedings of the VI International Symposium on Aquatic Oligochaetes held in Strömstat. Sweden. September 5–10. 1994 (pp. 199-206). Springer Netherlands.

Reynoldson. T. B., Rodriguez. P., Madrid. M. M. (1996). A comparison of reproduction. growth and acute toxicity in two populations of *Tubifex tubifex* (Müller. 1774) from the North American Great Lakes and Northern Spain. In Aquatic Oligochaete Biology VI: Proceedings of the VI International Symposium on Aquatic Oligochaetes held in Strömstat. Sweden. September 5–10. 1994 (pp. 199-206). Springer Netherlands.

Rocha. G. S., Tonietto. A. E., Lombardi. A. T., GG Melão. M. (2016). Effect of copper contaminated food on the life cycle and secondary production of *Daphnia laevis*. *Ecotoxicology and Environmental Safety*. 133. 235-242.

Rocha. O., Neto. A. J. G., dos Santos Lima. J. C., Freitas. E. C., Miguel. M., da Silva Mansano. A., Daam. M. A. (2018). Sensitivities of three tropical indigenous freshwater invertebrates to single and mixture exposures of diuron and carbofuran and their commercial formulations. *Ecotoxicology*. 27. 834-844.

Rossaro. B., Gaggino. G. F., & Marchetti. R. (1986). Accumulation of mercury in larvae and adults. Chironomus riparius(Meigen). Bulletin of environmental contamination and toxicology. 37(3). 402-406.

Sánchez-Moreno. S., Camargo. J. A., Navas. A. (2006). Ecotoxicological assessment of the impact of residual heavy metals on soil nematodes in the Guadiamar River Basin (Southern Spain). *Environmental Monitoring and Assessment*. 116. 245-262.

Shuhaimi-Othman. M., Nur-Amalina. R., & Nadzifah. Y. (2012). Toxicity of metals to a freshwater snail. Melanoides tuberculata. *The scientific world journal*. 2012.

Shuhaimi-Othman. M., Yakub. N., Ramle. N. A., & Abas. A. (2011). Toxicity of metals to a freshwater ostracod: Stenocypris major. Journal of Toxicology. 2011.

Shukla. O. S., & Murti. R. (1983). Cadmium and zinc toxicity to an aquatic insect. Ranatra elongata (Fabr.). Toxicology letters. 15(1). 39-41.

Sibley. P. K., Ankley. G. T., Cotter. A. M., & Leonard. E. N. (1996). Predicting chronic toxicity of sediments spiked with zinc: An evaluation of the acid-volatile sulfide model using a life-cycle test with the midge Chironomus tentans. Environmental Toxicology and Chemistry: An International Journal. 15(12). 2102-2112.

Slooff. W. (1982). A comparative study on the short-term effects of 15 chemicals on fresh water organisms of different trophic levels. RID-Mededelingen (Netherlands). no. 82-2.

Slooff. W. (1983). Benthic macroinvertebrates and water quality assessment: some toxicological considerations. *Aquatic Toxicology*. 4(1). 73-82.

Slooff. W., Canton. J. H., & Hermens. J. L. M. (1983). Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. I.(Sub) acute toxicity tests. Aquatic Toxicology. 4(2). 113-128.

Soundrapandian. S., & Venkataraman. K. (1990). Effect of heavy metal salts on the life history of Daphnia similis Claus (Crustacea: Cladocera). Proceedings: *Animal Sciences*. 99. 411-418.

Stuhlbacher. A., Bradley. M. C., Naylor. C., Calow. P. (1993). Variation in the development of cadmium resistance in Daphnia magna Straus; effect of temperature. nutrition. age and genotype. *Environmental Pollution*. 80(2). 153-158.

Sullivan. J. T., Palmieri. J. R., Agoes. R. (1977). Survival of *Lymnaea rubiginosa* infected with Echinostoma audyi and exposed to copper sulphate. Transactions of the Royal Society of Tropical Medicine and Hygiene. 71(1). 84-85.

Taylor. E. J., Maund. S. J., Pascoe. D. (1991). Toxicity of four common pollutants to the freshwater macroinvertebrates *Chironomus riparius* Meigen (Insecta: Diptera) and Gammarus pulex (L.)(Crustacea: Amphipoda). *Archives of environmental contamination and toxicology*. 21. 371-376.

Valenti. T. W., Cherry. D. S., Neves. R. J., Locke. B. A., & Schmerfeld. J. J. (2007). Case study: sensitivity of mussel glochidia and regulatory test organisms to mercury and a reference toxicant. *Freshwater Bivalve Ecotoxicology*.

Vedamanikam. V. J., & Shazili. N. A. M. (2009). The chironomid larval tube. a mechanism to protect the organism from environmental disturbances?. Toxicological & Environmental Chemistry. 91(1). 171-176.

Willford. W. A., & Cain. S. A. (1966). 18. Toxicity of 22 Therapeutic Compounds to Six Fishes.

Wurtz. C. B., Bridges. C. H. (1961). Preliminary Results From Macro—Invertebrate Bioassays. *Pennsylvania Academy of Science*. pp. 51-56.

Yılmaz. M., Gül. A., Karaköse. E. (2004). Investigation of acute toxicity and the effect of cadmium chloride (CdCl2· H2O) metal salt on behavior of the guppy (*Poecilia reticulata*). *Chemosphere*. 56(4). 375-380.

Zhu. X., Zhu. L., Chen. Y., & Tian. S. (2009). Acute toxicities of six manufactured nanomaterial suspensions to Daphnia magna. Journal of nanoparticle research. 11. 67-75.

Ziegenfuss. P. S., Renaudette. W. J., & Adams. W. J. (1986). Methodology for assessing the acute toxicity of chemicals sorbed to sediments: testing the equilibrium partitioning theory. Aquatic toxicology and environmental fate. 9. 479-493.

Supplementary Material – Chapter 4

Integrated Response of Biomarkers of stress and oxidative damage in sublethal exposures to different metals for the ostracod *Strandesia rondoniensis*

Table – S1 – Values of nominal concentrations, measurements and metal detection limits for the stock solutions used in toxicity tests in this study.

Stock solution concentration	Metal	Nominal Concentration (mg L^{-1})	Quantified Concentration (mg L^{-1})	Detection limit	Reference Method
10 mg L-1	Cd	6.1	6.69 ± 0.05	0.0006	SMWW 3111 B
	Cu	2.45	2.31 ± 0.08	0.003	SMWW 3111 B
	Zn	4.7	5.21 ± 0.01	0.002	SMWW 3111 B
	Hg	7.3	7.29 ± 0.2	2,000000E-6	USEPA 1631 E / USEPA 1630

Table – S2 – Average values and standard deviations of physical and chemical variables: pH, Dissolved Oxygen - O.D - (mg L^{-1}), Electric conductivity – C.E – (µs/cm) and temperature – T – (C^{o}), Hardness - ($CaCO_3$); measurements at the beginning and end of toxicity experiments subletals with ostracod *Strandesia rondoniensis*.

	Cu				Zn				Cd			Hg			
	initial														
	Control	0.001	0.01	0.1	Control	0.001	0.01	0.1	Control	0.001	0.01	Control	0.001	0.01	0.05
pH	7.47	7.68	7.58	7.7	7.56	7.62	7.71	7.77	7.57	7.6	7.72	7.89	7.88	7.88	7.78
O.D	6.99	6.7	6.2	6.8	5.3	5.1	4.9	4.8	4.3	6.95	4.9	6.6	6.8	6.4	6.7
T	24.5	24.6	24.3	24.5	25.5	25.4	25.3	25.3	25.1	25.2	25.3	24.3	24.3	24.3	24.7
C.e	133.4	145.3	149.6	143.4	137.5	142.1	139.2	142.2	152	149	147	172	177	173	168
Dureza	44	40	46	40	40	44	40	40	40	40	40	40	44	40	40
	Final														
	Control	0.001	0.01	0.1	Control	0.001	0.01	0.1	Control	0.001	0.01	Control	0.001	0.01	0.1
pH	7.5	7.81	7.91	7.84	7.8	7.5	7.57	7.3	7.86	7.9	7.84	7.9	7.93	7.8	7.83
O.D	5.4	6.3	5.9	6.4	7	6.84	6.72	6.63	6.82	5.4	7.08	6.4	5.8	5.6	5.3
T	24.4	24.6	24.8	24.7	25	24.7	24.7	25.5	26.3	7.08	26.1	25.3	24.5	25.3	25.2
C.e	185.6	187.9	184.8	186.3	212	199.2	216	206	201.3	210.1	213	189	182	180	182
Dureza	44	42	46	40	40	46	40	40	40	44	40	40	46	40	44

Table 3 – Protein values of the *Strandesia rondoniensis* ostracod samples, which were used to calculate the biomarkers.

Copper Sulfate			
Controle	0.001	0.01	0.1
3.791957	6.749751	5.51346	5.081422
4.084413	5.101363	8.318378	10.74111
6.095048	8.876703	7.367896	5.682951
4.725823	11.61848	8.344965	5.290794
5.400465	8.115653	9.381854	6.636756
Zinc Chloride			
Controle	0.01	0.1	1
3.157195	2.658691	3.436358	3.117315
3.376537	2.874709	3.944832	2.246594
2.133599	4.499834	4.911931	2.25324
4.582918	2.828182	2.854769	2.811565
2.465936	2.226653	2.824859	2.342971
Cadmium Chloride			
Controle	0.001	0.01	-
5.417082	2.196743	4.453307	-
5.52343	4.86208	3.113991	-
6.161515	3.456298	4.539714	-
3.68561	4.370223	3.742107	-
4.054503	4.795613	3.712197	-
Mercury Chloride			
Controle	0.001	0.01	0.05
7.141908	5.613161	4.659355	2.043868
6.218013	6.503822	5.214357	7.45098
3.306746	4.184114	3.253573	6.999003
6.048521	5.201063	4.393486	3.984713
7.490861	3.376537	2.246594	4.958458

Table 4 – Biomarker responses (MT – nmol mg pt^{-1}; LPO - nmol mg pt^{-1}; DNA-SB - µg DNA mg pt^{-1}; SOD – U mg pt^{-1}; GSH - nmol mg pt^{-1}), of *Strandesia rondoniensis* in sublethal exposures of 96 hours to metallic salts Cu, Zn, Cd e Hg.

	Cu				**Zn**				**Cd**			**Hg**			
	Control	0.001	0.01	0.1	Control	0.001	0.01	0.1	Control	0.001	0.01	Control	0.001	0.01	0.05
MT	0.008 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.008 ± 0.002	0.011 ± 0.004	0.019 ± 0.004	0.014 ± 0.005	0.024 ± 0.006	0.0132 ± 0.004	0.016 ± 0.004	0.02 ± 0.005	0.013 ± 0.002	0.02 ± 0.008	0.017 ± 0.005	0.025 ± 0.012
LPO	0.013 ± 0.023	0.028 ± 0.019	0.060 ± 0.018	0.044 ± 0.014	0.084 ± 0.016	0.117 ± 0.059	0.084 ± 0.171	0.171 ± 0.097	0.075 ± 0.036	0.088 ± 0.039	0.217 ± 0.187	0.046 ± 0.032	0.075 ± 0.008	0.109 ± 0.031	0.125 ± 0.062
DNA	14.25 ± 3.22	9.88 ± 3.29	11.57 ± 2.49	16.60 ± 4.16	16.9 ± 1.94	23.6 ± 7.44	16.6 ± 3.07	22.7 ± 4.88	9.94 ± 1.48	16.78 ± 2.95	18.44 ± 3.44	9.40 ± 1.49	11.43 ± 1.72	13.06 ± 3.59	14.23 ± 7.170
SOD	5.72 ± 0.163	5.32 ± 2.362	4.47 ± 2.33	7.49 ± 1.61	5.82 ± 0.995	5.85 ± 2.059	3.24 ± 1.029	5.25 ± 1.965	5.08 ± 0.946	7.38 ± 2.151	13.11 ± 4.431	4.98 ± 1.583	1.83 ± 0.745	6.83 ± 0.949	20.00 ± 10.11
GSH	0.002 ± 0.001	0.00097 ± 0.00067	0.002 ± 0.002	0.002 ± 0.0001	0.003 ± 0.0006	0.003 ± 0.001	0.003 ± 0.001	0.006 ± 0.002	0.0017 ± 0.001	0.004 ± 0.002	0.007 ± 0.004	0.003 ± 0.001	-	0.005 ± 0.002	-