



Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais



Efeitos da contaminação por elementos traço em riachos: uma abordagem com diferentes grupos tróficos funcionais de insetos aquáticos

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Orientador: Prof. Dr. Luiz Ubiratan Hepp

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RESUMO

O aumento nas concentrações de metais vem sendo considerado um grave problema de poluição ambiental nos ambientes aquáticos continentais, pela sua persistência, toxicidade e capacidade de ser incorporado nos organismos. Neste contexto, procurei entender como o aumento das concentrações de cobre e cádmio afetam os grupos tróficos funcionais de insetos em riachos. Assim, os trabalhos desenvolvidos nesta tese tiveram como objetivos (i) verificar os efeitos das concentrações de cobre (Cu) e cádmio (Cd) nas interações ecológicas entre insetos aquáticos e o meio biótico e abiótico, (ii) verificar os efeitos do aumento das concentrações de Cu e Cd em diferentes rotas tróficas de insetos aquáticos, e (iii) observar o comportamento alimentar de insetos aquáticos sobre diferentes concentrações de Cu e Cd. Para alcançar estes objetivos realizei diferentes experimentos, em campo e laboratório, considerando diferentes rotas tróficas de insetos (i.e. raspadores/herbivoria, fragmentadores/detrítivoros e predadores/predação). Diante dos resultados observados, conclui que (i) a intensidade de atividades antrópicas (especialmente agricultura), na área de drenagem dos riachos contribui para o aumento das concentrações de Cu e Cd no sedimento e coluna d'água dos riachos, refletindo nas quantidades de metais incorporadas por insetos raspadores a partir do consumo de recursos alimentares; (ii) a presença de concentrações de metais na água reduziu drasticamente as taxas de alimentação de insetos fragmentadores e, consequentemente, afetou negativamente o processo de decomposição foliar e; (iii) o aumento nas concentrações de Cu e Cd afeta negativamente a interação predador-presa, uma vez que reduziu a mobilidade, a procura e o sucesso de captura de presas pelos predadores. Assim, concluí que a presença de metais no ambiente aquático pode afetar negativamente as rotas alimentares de insetos aquáticos, podendo refletir na diminuição da sua biodiversidade e prestação de serviços ambientais. Ainda, considerando que os insetos aquáticos são organismos que compreendem um nível intermediário da cadeia trófica aquática, a contaminação destes organismos pode afetar os níveis tróficos superiores. Desta forma, os resultados obtidos nesta tese auxiliam na formação de uma base conceitual sólida sobre os efeitos que os metais podem causar ao ambiente aquático, subsidiando, assim, a elaboração de estratégias de mitigação dos efeitos antrópicos sobre a biota aquática e processos ecológicos.

Palavras-chave: cobre; cádmio; fragmentadores; raspadores; predadores; comportamento biológico.

ABSTRACT

The increase in metal concentrations has been considered a serious environmental pollution problem in continental aquatic environments, due to its persistence, toxicity and ability to be incorporated into organisms. In this context, I looked to understand how the increase in copper and cadmium concentrations affect the functional feeding groups of insects in streams. Thus, the work carried out in this thesis aimed at (i) verifying the effects of copper (Cu) and cadmium (Cd) concentrations on the ecological interactions between aquatic insects and the biotic and abiotic environment, (ii) verifying the effects of increased concentrations of Cu and Cd in different trophic routes of aquatic insects, and (iii) observe the feeding behavior of aquatic insects under different concentrations of Cu and Cd. To achieve these goals, I carried out different experiments, in field and laboratory, considering different trophic routes of insects (ie scrapers / herbivory, shredders / detritivores and predators / predation). In view of the observed results, I conclude that (i) the intensity of anthropogenic activities (especially agriculture), in the streams drainage area contributes to the increase of the concentrations of Cu and Cd in the sediment and water column, reflecting in the amounts of metals incorporated by scraper insects from the consumption of food resources; (ii) the presence of concentrations of metals in water drastically reduced the feeding rates of shredders and, consequently, negatively affected the leaf decomposition process; (iii) the increase in Cu and Cd concentrations negatively affects the predator-prey interaction, since it reduced mobility, foraging and the success of prey capture by predators. Thus, I concluded that the presence of metals in the aquatic environment can negatively affect the food routes of aquatic insects, which may reflect on the decrease of biodiversity and provision of environmental services. Also, considering that aquatic insects are organisms that comprise an intermediate level of the aquatic food chain, the contamination of these organisms can affect higher trophic levels. In this way, the results obtained in this thesis help in the formation of a solid conceptual base on the effects that metals can cause to the aquatic environment, thus subsidizing the elaboration of strategies to mitigate the anthropogenic effects on aquatic biota and ecological processes.

Key-words: copper; cadmium; shredders; scrapers; predators; biological behavior.

APRESENTAÇÃO

Esta Tese apresenta uma Introdução Geral contendo as principais informações conceituais sobre o tema em estudo, bem como o problema que motivou a realização dos trabalhos. Na sequência, apresenta quatro capítulos em formato de manuscritos científicos, os quais buscaram atender aos objetivos propostos pela tese. O primeiro capítulo, intitulado “Stream contamination by trace elements: biota incorporation and phytoremediation” foi realizada uma revisão bibliográfica que apresenta informações sobre os problemas causados pelos metais em riachos e estratégias de bioremediação. Por fim, apresento Considerações Finais e Perspectivas com as principais observações desta tese e sugestões de desdobramento da linha de pesquisa. As citações ao longo do texto e referências bibliográficas dos capítulos seguem as normas propostas pelos periódicos propostos. O segundo capítulo, intitulado “The influence of the environment in the incorporation of copper and cadmium in scraper insects” foi desenvolvido a partir de um experimento de campo e teve por objetivo principal verificar se os usos do solo e as concentrações de Cu e Cd no ambiente aquático influenciam as concentrações de Cu e Cd em duas espécies de insetos raspadores. A conclusão principal desse artigo foi que embora as características funcionais sejam importantes para o entendimento do funcionamento dos ecossistemas, neste estudo, constatamos que as características fisiológicas podem ser determinantes nas concentrações de metais em insetos aquáticos. No terceiro capítulo, intitulado “Effects of copper and cadmium on stream leaf decomposition: evidence from a microcosm study”. Neste estudo foi realizada uma abordagem em microcosmo, com o objetivo principal de avaliar os efeitos dos metais Cu e Cd no condicionamento microbiano e no consumo de detritos foliares por fragmentadores em condições controladas. A conclusão principal desse artigo foi que o aumento das concentrações de Cu e Cd reduziu o consumo de serapilheira por *Phylloicus* sp. em até 60%. Como consequência, foi observado uma diminuição de $\sim 3\times$ nas taxas de decomposição nos tratamentos com os metais. No quarto capítulo, intitulado “Mudanças comportamentais em insetos predadores na presença de metais”, o objetivo foi avaliar o comportamento do predador *Lestes* sp. durante a captura de presas em condições de microcosmo. Foram avaliados o tempo de captura da presa, procura, tentativas de captura e mobilidade pelas larvas sob diferentes cenários de contaminação de Cu e Cd. A conclusão principal desse artigo foi que o aumento das concentrações de cobre e cádmio no ambiente afetam o comportamento de predação de *Lestes* sp.

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INTRODUÇÃO GERAL

A água é um elemento essencial para a manutenção da vida na terra. No entanto, nos últimos anos, os ecossistemas aquáticos continentais vêm sofrendo uma série de ameaças associadas às atividades humanas, como aporte de efluentes industriais, expansão das atividades agrícolas, problemas de gestão de resíduos urbanos e aumento na urbanização (HÄDER et al., 2020). Uma quantidade expressiva de poluentes como herbicidas, pesticidas, fertilizantes e outros produtos químicos potencialmente perigosos da agricultura e da indústria são despejados nos corpos hídricos diariamente (CORBI et al., 2010). Estes produtos podem causar danos primários, como efeitos deletérios na fauna e flora aquática e danos secundários, na forma de perturbações no equilíbrio da teia alimentar biológica em que os efeitos acabam sendo potencializados ao longo do tempo (SUMUDUMALI; JAYAWARDANA, 2021).

Outro fator chave para a qualidade dos ecossistemas aquáticos é a relação com os usos e ocupação do solo nas áreas adjacentes a esses ambientes (RAMIÃO; CÁSSIO; PASCOAL, 2020). Alterações na paisagem constituem um problema ambiental importante para a integridade dos ecossistemas aquáticos (ALLAN, 2004; ROVANI et al., 2020). Em riachos, por exemplo, as zonas ripárias exercem um papel fundamental na manutenção do estado ecológico dos riachos (BURDON et al., 2020). A presença de vegetação natural na zona ripária auxilia na regulação da temperatura do riacho através do sombreamento da insolação solar, estabilização das margens, fornecimento de habitat e alimento para uma variedade de organismos terrestres e aquáticos (SINGH; TIWARI; SINGH, 2021; TONIN et al., 2017), refletindo assim, nas características físicas, químicas e biológicas do ambiente (BURDON et al., 2020).

O crescimento industrial e a urbanização, associados com o crescimento populacional, vêm alterando os processos naturais em riachos, especialmente pela retirada da vegetação ripária (HEPP et al., 2016) e pela contaminação do solo (HUNT et al., 2016). Os rios e riachos são fontes estratégicas de água para uso doméstico e para apoiar projetos de desenvolvimento econômico, como irrigação, geração de energia hidrelétrica e mineração (CAPPS; BENTSEN; RAMÍREZ, 2016). Como resultado do uso irresponsável da água para suprir a demanda populacional, estas atividades acabam sendo importantes fontes poluidoras dos recursos hídricos (CAPPS; BENTSEN; RAMÍREZ, 2016; WEERAPRAPAN et al., 2015).

Neste sentido, o aumento da contaminação aquática por metais potencialmente tóxicos vem se tornando um problema ambiental cada vez mais reportado (CORBI et al., 2010;

FRANCISCO et al., 2019; GABRIEL et al., 2020; HEPP; PRATAS; GRAÇA, 2017). Os metais são elementos de ocorrência natural e são definidos por ter uma densidade alta em relação à água (QADRI et al., 2020). Os metais são constituintes da crosta terrestre e sua presença está associada com intemperismo das rochas e do solo, assim como é influenciada por fenômenos climáticos (MAYER-PINTO et al., 2010; WANG et al., 2016). O crescimento populacional aumentou consideravelmente as concentrações destes elementos no solo, atmosfera e nos ambientes aquático como resultado de uma demanda de seu uso em diversas aplicações industriais, agrícolas, domésticas e tecnológicas (SILVA; SILVEIRA; CAMPOS, 2017; WANG et al., 2016). Assim, nos últimos anos, tem havido uma crescente preocupação ecológica e de saúde pública global devido à sua natureza tóxica (FRANCISCO et al., 2019).

Nos ambientes aquáticos, os metais distribuem-se em diferentes compartimentos ambientais, como na água, no sedimento e na biota (MAHMOOD et al., 2018; PROMMI; PAYAKKA, 2018). O sedimento é considerado um importante compartimento na acumulação de metais no ambiente, pois incorpora os poluentes através da adsorção, tornando os menos biodisponíveis (WILLIS; BISHOP, 2016). Além de acumular os metais, este compartimento pode reprocessar esses elementos para a coluna d'água tornando-os disponíveis novamente ao meio (NYSTRAND et al., 2012), assim, os efeitos podem persistir para a biota aquática ao longo de diversas gerações.

Quando associado a biota aquática, os metais podem ser classificados como essenciais e não essenciais (MAYER-PINTO et al., 2010). O primeiro grupo consiste nos elementos essenciais (e.g. cobre, zinco, ferro, manganês), os quais desempenham papel importante no metabolismo dos organismos (LEBRUN et al., 2014; Mayer-Pinto et al., 2010). Porém, em níveis superiores aos valores fisiológicos, os metais essenciais podem exercer efeitos negativos sobre os organismos e o meio ambiente (ADAMS et al., 2020; RAINBOW, 2002). No segundo grupo estão os elementos não essenciais (e.g. arsênio, cádmio, chumbo, mercúrio, níquel), os quais não exercem função bem definida para a biota aquática, podendo ser prejudiciais mesmo em baixas concentrações (RAINBOW, 2002). A contaminação por metais é prejudicial para os organismos individualmente e também para populações e comunidades expostas (CUI et al., 2018; ZHANG et al., 2018).

De modo geral, tanto metais essenciais quanto não essenciais podem ser potencialmente tóxicos aos organismos, porém, no caso dos essenciais, a toxicidade ocorre em situações de deficiências metabólicas ou em concentrações altas, acima de limites fisiológicos ou dependendo do seu estado de valência (SFAKIANAKIS et al., 2015). Individualmente os metais podem causar problemas no crescimento, maturação, reprodução, além de problemas

comportamentais (MOGREN; TRUMBLE, 2010). No ambiente, o aumento da concentração de metais pode gerar desequilíbrios às populações e comunidades expostas, comprometendo os ecossistemas naturais (HEPP; PRATAS; GRAÇA, 2017).

O cobre é um metal que a princípio não é um problema no meio ambiente (i.e. solo, ar e água), pois é um micronutriente e faz parte do metabolismo dos organismos vivos (HADJIPANAGIOTOU et al., 2020; HUNTING et al., 2013). No entanto, quando em altas concentrações e na forma biodisponível, isto é, quando os íons de cobre estão prontamente disponíveis para interagir com os organismos, este elemento pode ser um problema sério para a saúde pública e para o meio ambiente (MAGALHÃES et al., 2015). O cobre vem sendo utilizado em larga escala na agricultura como fungicida, bactericida e herbicida (REHMAN et al., 2019). Quando em concentrações elevadas no ambiente o cobre pode causar efeitos negativos, como por exemplo, reduzindo a diversidade de invertebrados e fungos aquáticos (HUNTING et al., 2013). Em concentrações subletais, o cobre pode gerar efeitos negativos aos organismos, como a redução na taxa de crescimento e intensidade de respiração (GOLOVANOV, 2008).

Por sua vez, o cádmio é um elemento não essencial, com um potencial altamente tóxico. Em ambientes naturais, as rochas ígneas são responsáveis pelas baixas concentrações de cádmio no ambiente (MAR; OKAZAKI, 2012). Por outro lado, em ambientes com influência antrópica, mais de 90% das fontes cádmio são oriundas de fertilizantes fosfatados e de deposição atmosférica (PAN et al., 2010). O cádmio apresenta propriedades químicas semelhantes ao zinco (DIETRICH et al., 2016) e em alguns casos, pode substituir elementos essenciais e interferir no funcionamento de enzimas dos organismos (TORRES et al., 2008). Além disso, a exposição ao cádmio pode alterar a expressão proteica em espécies de insetos aquáticos, alterando seu desenvolvimento (LEE et al., 2006).

Riachos, por possibilitarem estudos da dinâmica entre os diferentes compartimentos se tornam locais interessantes para explorar estratégias de conservação e restauração em um ecossistema contaminado por metais (SCHULTHEIS; SANCHEZ; HENDRICKS, 1997). Algas, insetos e peixes são considerados bons modelos biológicos para estudos com poluentes em ecossistemas aquáticos (STANKOVIC; KALABA; STANKOVIC, 2014). A utilização de invertebrados como modelo de estudo, vem demonstrando resultados satisfatórios, uma vez que as formas de coletas e utilização desses organismos são bem descritas na literatura (MEBANE; SCHMIDT; BALISTRERI, 2017; RODRIGUEZ et al., 2018). Ainda, esses organismos apresentam um ciclo de vida rápido, podendo observar-se resultados importantes em um curto período de tempo (CAYROU; CÉRÉGHINO, 2005).

As respostas biológicas aos metais são determinadas pelas concentrações que são assimiladas pelo organismo (HARE, 1992). Além disso, os efeitos dos metais são influenciados pelas características individuais de cada elemento químico (e.g. especiação química). A absorção dos metais do meio externo pelos organismos pode ocorrer a partir da assimilação direta do meio (dissolvido na água), ou da assimilação indireta via dieta (ADAMS et al., 2011). Assim, os organismos são expostos a metais por diferentes compartimentos limnológicos e, dependendo da organização estrutural e funcional de um ecossistema, a exposição ao metal pode ocorrer por meio de diferentes rotas (por exemplo, alimento ou água) ou uma combinação de diferentes fontes (CAIN; CROTEAU; LUOMA, 2011).

As concentrações de cobre acumuladas nos organismos podem variar conforme o tipo de recurso utilizado pelos organismos (LIESS; GERNER; KEFFORD, 2017; LOUREIRO et al., 2018). No entanto, os efeitos negativos são observados, principalmente nos consumidores primários (e.g. herbívoros e detritívoros) (HOGSDEN; HARDING, 2012). Por sua vez, o cádmio pode afetar o condicionamento em folhas e a diversidade de fungos em riachos, alterando as taxas de alimentação por organismos detritívoros e, conseqüentemente, o funcionamento dos ecossistemas aquáticos (CAMPOS et al., 2014). Portanto, os metais podem afetar diferentes grupos alimentares de insetos em riachos (MENEGAT et al., 2020; PASTORINO et al., 2020), o que irá refletir em alterações na biodiversidade e funcionamento desses ambientes.

As estratégias alimentares dos insetos aquáticos são características que acabam refletindo nas adaptações às condições do ambiente (TOMANOVA; GOITIA; HELEŠIC, 2006). As rotas de alimentação iniciam-se por duas fontes distintas (alóctone e autóctone) (CUMMINS, 2016). A rota de alimentação com fonte basal alóctone, representada principalmente pelo consumo de folhas (detrito) oriundas da vegetação ripária (GRAÇA et al., 2015), é iniciada basicamente por organismos fragmentadores (BOYERO et al., 2016). Por outro lado, a rota de alimentação de fonte basal autóctone, geralmente representada pelo biofilme (GREGORY et al., 1987), constitui a principal fonte de alimento para os organismos raspadores (COLLINS et al., 2016). As partículas finas que restam do processo de alimentação das duas rotas de alimentação são utilizadas como recursos por organismos coletores-catadores e coletores-filtradores (CUMMINS, 2016). E por fim, os insetos predadores, geralmente definidos como predadores intermediários, por se alimentar de invertebrados e pequenos vertebrados e também ser um importante recurso alimentar para organismos maiores (e.g. peixes) (KALKMAN et al., 2008).

Uma vez tendo o alimento como principal fonte de incorporação de metais, as rotas tróficas de invertebrados ou intermediadas por esses organismos podem causar alterações significativas na estrutura trófica em riachos (HEPP; PRATAS; GRAÇA, 2017). Em riachos, as fontes de energia podem ser algas perifíticas (fonte autóctone) ou detritos vegetais (fonte alóctone). No entanto, estas fontes de energia são facilmente influenciadas por estressores antrópicos, refletindo negativamente no funcionamento dos ecossistemas (FERREIRA et al., 2016). Assim, os impactos sobre o funcionamento dos riachos dependem não apenas da riqueza e composição das espécies, mas também das interações da teia alimentar (CLEMENTS, 2017; HOGSDEN; HARDING, 2012; RODRIGUEZ et al., 2018).

Estudo que avaliam a incorporação e acumulação de metais por diferentes grupos tróficos de insetos aquáticos são, de forma geral, bem documentados na literatura (CAIN; CROTEAU; LUOMA, 2011; CORBI et al., 2011; GRIBOFF et al., 2018; PASTORINO et al., 2020; XIN et al., 2015). No entanto, o que se observa é que as especificidades relacionadas a incorporação dos metais por determinados grupos tróficos de insetos aquáticos (e.g. raspadores e fragmentadores) ainda é incipiente. Esses dois grupos tróficos estão muito associados aos aspectos funcionais dos ambientes aquáticos, em especial, de riachos, pois são os primeiros organismos a processarem a matéria orgânica basal da cadeia trófica. Ao consumirem os produtores primários, os insetos raspadores estão dando início a transformação e transferência de matéria pela cadeia trófica (CUMMINS, 2016; RODRIGUEZ et al., 2018). Por outro lado, os insetos fragmentadores, juntamente com os fungos aquáticos, iniciam o processamento da matéria orgânica alóctone que será transformada em partículas menores e servirá de alimento para outros grupos tróficos (e.g. filtradores e coletores) (GRAÇA et al., 2015).

Nesta Tese, a partir de estudos com diferentes abordagens metodológicas (i.e. campo e laboratório) e revisão de literatura, tento elucidar os efeitos da incorporação de metais por diferentes grupos tróficos de insetos aquáticos (e.g. raspadores, fragmentadores e predadores) sobre o comportamento, distribuição de organismos e funcionamento dos ecossistemas. Optei por estudar mais detalhadamente insetos raspadores, fragmentadores e predadores por entender que estes organismos fazem parte de níveis tróficos estratégicos em riachos. Enquanto os raspadores e fragmentadores são responsáveis pela incorporação e transformação das diferentes fontes de energia nos riachos, os predadores possuem um papel de consumidores em níveis superiores na cadeia alimentar dos insetos aquáticos.

Objetivos

Diante dos elementos apresentados anteriormente, os objetivos principais desta tese são:

- verificar os efeitos das concentrações de cobre e cádmio nas interações ecológicas entre insetos aquáticos e o meio biótico e abiótico;
- verificar os efeitos do aumento das concentrações de cobre e cádmio em diferentes rotas tróficas de insetos aquáticos;
- observar o comportamento alimentar de insetos aquáticos sobre diferentes concentrações de Cu e Cd.

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CAPÍTULO 1

Stream contamination by trace elements: biota incorporation and phytoremediation



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Stream contamination by trace elements: biota incorporation and phytoremediation

Abstract

In this review we present information about the presence of trace elements on stream and the problems that the increase these chemical elements can cause on aquatic systems. Although several studies on trace elements contamination in aquatic environments are conducted contamination sources, *non-point* sources have higher potential to contaminate water bodies. Some trace elements are important for the development of organism, however, high concentrations makes them toxic to aquatic organisms. In some cases, trace elements may accumulate and be transferred along food webs, generating changes in the structure and functioning of terrestrial ecosystems. The phytoremediation techniques could be an important tool in reducing the problems generated by trace elements. Thus, understanding the effects generated by the increase of trace elements concentrations on aquatic ecosystems becomes important to help public managers to show solutions for the mitigation of natural resources.

Key-words: bioaccumulation, biomagnification, heavy metals, metalloids.

Resumo

Nesta revisão apresentamos algumas informações sobre a presença dos elementos traço em riachos e os problemas que o aumento destas concentrações podem causar para estes ambientes. Apesar de grande parte dos estudos sobre contaminação de elementos traço em ambientes aquáticos serem realizados em fontes pontuais de contaminação, as fontes não pontuais têm potencial para contaminar os corpos hídricos. Alguns elementos traço são importantes para o desenvolvimento dos organismos, no entanto, o aumento das concentrações o torna tóxico para os organismos aquáticos. Em alguns casos os elementos traço podem acumular e ser transferidos ao longo das redes alimentares, gerando alterações na estrutura e funcionamento destes ambientes. As técnicas de fitorremediação podem se tornar importantes ferramentas na redução dos problemas gerados por estes elementos. Assim, compreender os efeitos gerados pelo aumento nas concentrações de elementos traço nos ecossistemas aquáticos se torna importante para auxiliar os gestores públicos a buscar soluções para a mitigação dos recursos naturais.

Palavras-chave: bioacumulação; biomagnificação; metais; metalóides.

1. Introduction

The agriculture modernization with the aim of improve productivity has considerably increased the replacement of native vegetation for cultivated areas and, consequently, the use of agrochemicals (Foley et al., 2011). In 2015, in Brazil, approximately 899 million liters of pesticides were used in 71.2 million hectares of crops, with soybeans, the crop with the largest planted area averaging 17.7 l/ha (Pignati et al., 2017). The removal of native vegetation by agriculture practices is responsible for changes in terrestrial environments (Molina et al., 2017), such as soil erosion, changes in biogeochemical cycles (Lemaire et al., 2014) and soil contamination (Atafar et al., 2010). Fertilizers and pesticides used in crops contain high concentrations of trace elements, such as lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), arsenic (As), copper (Cu), zinc (Zn), and others (Nziguheba & Smolders, 2008; Atafar et al., 2010). Initially, trace elements are responsible for soil contamination of cultivable areas (Yang et al., 2013), and later can easily be carried to water bodies, increasing sediment and water concentrations (Corbi et al., 2008).

Soybeans are the fastest growing crop in the world, Brazil being the world's second largest producer (Martins et al., 2018). Thus, the use of phosphate fertilizers is one of the main strategies for the best crop yield (Caires et al., 2017). The use of phosphate fertilizers in soybean crops has become a source of accumulation of trace elements for agricultural land use. Phosphate rocks used in the formulation of phosphate fertilizers may have trace elements such as Cu, Cd and As (He et al., 2005). In addition, the presence of trace elements in agricultural practices may be associated with the edaphic characteristics (Marrugo-Negrete et al., 2017) and other fertilizers and pesticides used in crops (Kelepertzis, 2014).

The increase in trace element concentrations has been considered a serious problem of environmental pollution in aquatic environments, specially river and streams (Stankovic et al., 2014). These problems focus on their toxicity and capacity of incorporation by the organisms (Rogel et al., 2004; Teng et al., 2015). In view of this problem, strategies for the removal of trace elements from the environment have been used to mitigate effects on the environment (Lee, 2013). Different techniques for remediation have been used *in situ* to immobilize trace elements in the environment, such as amendements, sand cap and phytoremediation (Peng et al., 2009). The phytoremediation consists in a plant natural ability to remove, reduce and/or degrade toxic elements in the environment (Peng et al., 2009). The phytoremediation has been considered a good approach in reducing trace element concentrations (Pratas et al., 2014;

Demarco et al., 2018), because of its low cost and because it is an ecologically correct approach (Lee, 2013).

In this sense, knowledge increasing about the effects of agriculture on aquatic environments, especially on trace elements contribution in these ecosystems, is important for decisions making about the water resources management. In this work, we showed information from studies and researches on trace elements toxicity on aquatic organisms, considering the different incorporation forms of these elements into the aquatic trophic chain and eventual transference to terrestrial environment. In addition, we will present comments about possible approaches about aquatic environments remediation, as a way to mitigate these effects and guide public managers. Our focus will be on small streams, since about 2/3 of the river basin is drained by these environments (Allan & Castillo, 2007). Also, considering areas of extreme agricultural activity, the upper river basins become susceptible to this type of impact.

2. Trace elements on aquatic environments

Trace elements mean elements present at low concentrations in the ecosystems. The trace elements may be present in aquatic environments in a natural and/or anthropogenic way (Rainbow, 2002). In natural environments, trace element concentrations are controlled by geological factors, such as rock weathering (Magalhães et al., 2015), and generally do not affect the environment as they are present in low concentrations. On the other hand, in environments impacted by anthropic activities, the main sources of contamination of these elements are mining, industrialization, urbanization and agriculture (Xu et al., 2014).

The streams contamination influenced by agricultural activities has been investigated in recent years (Szöcs et al., 2017; Kroflič et al., 2018; Loureiro et al., 2018). The formulation of fertilizers and pesticides is associated with a large variety of trace elements (Atafar et al., 2010). Thus, the compounds concentrations responsible for environmental contamination may vary according to soil type, crop and forms of management used (He et al., 2005).

In the aquatic environment, the trace element distribution is associated with the physical properties and chemical speciation of each element. In general, trace elements may be associated with fractions of different sizes, such as the dissolved fraction ($1 < \text{kDa}$), colloidal ($1 \text{ kDa} - 0.45 \mu\text{m}$) and particulate ($> 0.45 \mu\text{m}$) (Nystrand et al. 2012). The partition of trace elements under different forms depends mainly on temperature, pH and redox potential (Aguilar-Hinojosa et al., 2016). The limnic sediment is an important reservoir of

trace elements, where changes in the water physical and chemical characteristics can mobilize and release trace elements to the water column (Montalvo et al., 2014) making them available to the organisms.

The chemical speciation of the trace elements are the conditions in which the elements are in the environment. The trace elements are found in the environment in a variety of forms, including organic and inorganic complexes and hydrated free ions (Slaveykova & Wilkinson, 2005). The trace element forms derived from the speciation processes that determine their availability and toxicity to the aquatic biota. Arsenic, for example, is mainly found on the trivalent (AsIII) and pentavalent forms (AsV), although the two forms are considered toxic, the trivalent form can be considered more toxic (Singh et al., 2015).

3. Incorporation and mobility of trace elements by aquatic invertebrates

When related to aquatic biota, trace elements are classified in two categories; the first group consists on the essential elements (e.g., Cu, Zn, and Mn). The elements of this group play an important role on the organisms metabolism (Lebrun et al., 2014). However, they can also produce toxic effects when absorbed and / or adsorbed in excess by organisms (Tüzen, 2009). Zinc, for example, is an important component for some enzymes (e.g., metalloproteinases, peptidases and carbonic anhydrase). The copper is present mainly in the respiratory protein hemocyanin (Rainbow, 2002). The second group is the non-essential elements (e.g., As, Cd, Pb, Hg, Ni). These elements do not exert a well-defined function for aquatic biota, and may be toxic even at low concentrations (Torres et al., 2008). Non-essential elements when accumulated can affect growth and reproduction rates of aquatic organisms (Adams et al., 2011).

Aquatic invertebrates include different *taxa* of aquatic and semi-aquatic organisms that play an important role in the food chain, transforming organic matter in fine particles and serving as a food resource for other vertebrate organisms. For aquatic invertebrates, exposure to metal may occur from adsorption by their exoskeleton and/or by feed (Magalhães et al., 2015). The incorporation of the trace element by adsorption occurs in direct contact with the environment (e.g., stream water). This incorporation form is common when organisms live in environments with higher concentrations of trace elements, or when they remain for a long time exposed to the element (Hepp et al., 2017). On the other hand, incorporation by food consumption is the most common form, since invertebrates are effectively in the aquatic food chain (Figure 1; Loureiro et al., 2018).

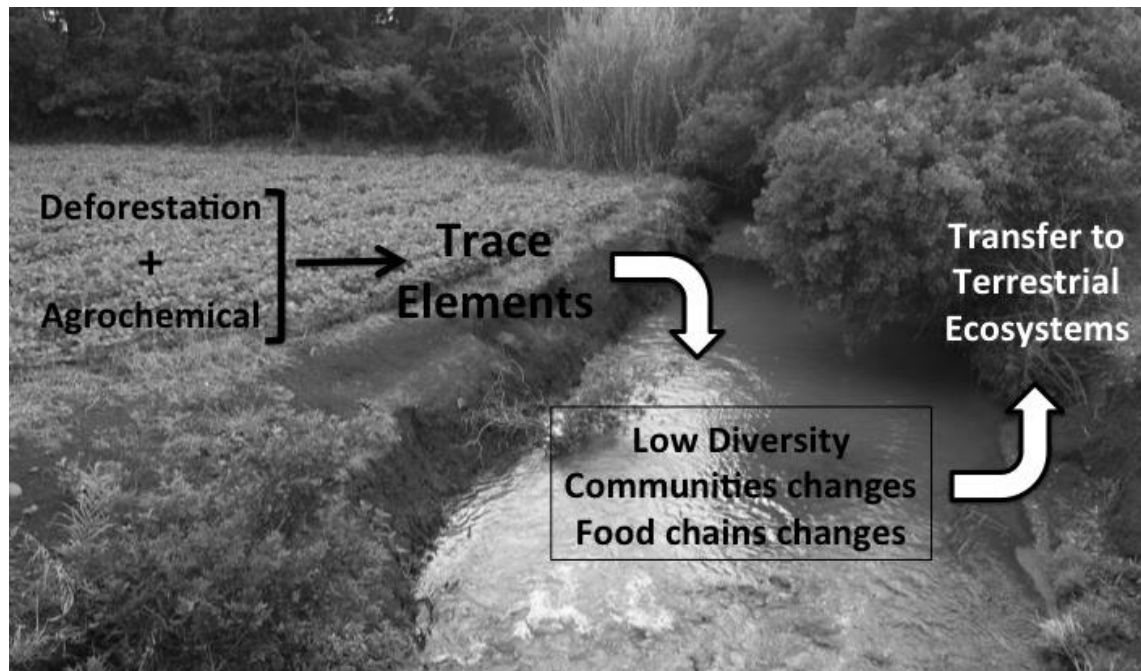


Figure 1. Route of contamination in streams/riparian zone by trace elements from agricultural practices and their transfer to the terrestrial ecosystems.

When incorporated by the organism, the destiny of the trace element is associated with processes of metabolism and/or excretion (Rainbow, 2002). Essential or non-essential trace elements accumulated can be transferred to a biologically inactive compartment and need to be excreted to avoid toxic effects on the organism (Rainbow, 2002). The exoskeleton storage of trace elements may be a strategy for their regulation and later elimination, from the ecdysis process (Keteles & Fleeger, 2001; Mogren et al., 2012).

The ability to incorporate and excrete trace elements can determine the potential for bioaccumulation of each element in organisms. The bioaccumulation occurs when the rates of incorporation are stored faster than they are metabolized or excreted (Luoma & Rainbow, 2005). In turn, bioaccumulation may vary according to the organism feed strategy (Rodriguez et al., 2018). For example, trace element concentrations in the biofilm may reflect the anthropization degree of the surrounding streams (Ancion et al., 2013). The biofilm is a major source of food for primary consumers (e.g., scrapers; Collins et al., 2015), and may be an important source of trace element incorporation in the trophic chain (Loureiro et al., 2018).

In addition to the ability of the trace element to accumulate in organisms, biomagnification is another important mechanism for incorporating contaminants into the aquatic food chain. While bioaccumulation is associated with the body's ability to accumulate trace elements by absorption/ingestion over time, biomagnification refers to trace element transference to higher levels of the trophic chain. Because trace elements have different

chemical characteristics, they show different behaviors when incorporated into the food chain. In general, organisms develop physiological strategies for the elements excretion, so they are hardly biomagnified, as is the case of arsenic, for example (Hepp et al., 2017). On the other hand, there are trace elements that are biomagnified, because the organisms do not have clear excretion strategies (e.g., mercury; Schmitt et al., 2011). In general, bioaccumulation occurs in a similar way in impacted environments and is independent of the trace element. On the other hand, biomagnification is exclusively dependent on the chemical characteristics of the trace element and is highlighted because it is a real form of human contamination.

The aquatic invertebrates can be an important source of contamination for terrestrial environments through food routes (Alberts et al., 2013). This is because, food routes linking terrestrial and aquatic environments through the organisms movement (Schindler & Smits, 2017). Aquatic invertebrates are important sources of resources for aquatic (e.g., fish) and terrestrial (e.g., birds) vertebrates (Alberts et al., 2013), which can subsidize both terrestrial and aquatic networks (Kraus et al., 2014).

Most of the aquatic insects has an immature phase on the aquatic environment and the adult phase on the terrestrial environment (Jonsson & Stenroth, 2016). Thus, the emergency process is a common step in the development of numerous insects species. In this way, considering the insects ability to incorporate trace elements in their immature phases, the emergence of these elements may be a real source of contamination to the terrestrial environment (Figure 1; Hepp et al., 2017). An example is the larvae of *Leptocerus americanus* (Banks, 1899) that when emerged from streams were able to transfer about 160 mg of lead to the terrestrial environment in a year (Ryan et al., 2019). In addition, emerging insects correspond to the main source of energy for birds, spiders and raccoons in riparian zones (Kautza & Sullivan, 2016), that is, they are quickly incorporated into the terrestrial trophic chain.

4. Phytoremediation of streams trace elements

Strategies for the remediation of aquatic environments contaminated by trace elements have been discussed in recent years (Lee, 2013; Favas et al., 2014). The phytoremediation has been considered an ecologically efficient alternative for the reduction of trace elements in the environment (Lee, 2013). In addition, the phytoremediation process consists in the use of organisms with high capacity for compounds accumulation (e.g., fungi, mosses, aquatic macrophytes; see the reviews Peng et al., 2009; Wasi et al., 2013; Akcil et al.,

2015). The selected species must be hyperaccumulating and are involved in the absorption, translocation, sequestration and degradation of trace elements available on the environment (Favas et al., 2014).

The use of phytoremediation in the recovery of aquatic environments can be classified in different approaches, which vary according to how the phytoremediative species incorporates the chemical elements: (1) *Phytoextraction* consists in the trace elements absorption by the root, being later translocated and accumulated in the aerial parts (Favas et al., 2014). This approach is the best procedure to remove contaminants from the water bodies sediment (Singh et al., 2012). (2) *Rhizofiltration* is defined as the ability of plants to absorb and accumulate trace elements in their roots (Singh et al., 2012). This procedure can be used in environments with low trace element concentrations (Lee, 2013). (3) *Phytostabilization* is the process that decreases the bioavailability and mobility of trace elements in the environment (Singh et al., 2012). Phytostabilization can be used in contaminated soils near streams, where vegetation can reduce erosion and leach contaminants to the groundwater (Lee, 2013). This process does not degrade the trace element, however, it reduces the compound ability to migrate to soil and water (Lee, 2013). (4) *Phytovolatilization* consists in the ability of certain plants to absorb the trace elements by the root transforming into non-toxic forms and dispersing to the atmosphere from the volatilization (Favas et al., 2014).

The use of phytoremediation plants is a recent approach and the literature lacks information about which species have the best efficiency in trace elements removal (Lee, 2013). However, these approaches have presented some advantages and disadvantages (Table 1), depending on the plants efficiency in to absorb the elements under certain conditions. In an *ex situ* study testing the phytofiltration potential of three species of aquatic macrophytes (*Callitriche stagnalis* Scop., *Potamogeton natans* L. and *P. pectinatus* L.), Pratas et al. (2014) found an approximately 85% of reduction in Uranium concentrations in water. Demarco et al. (2018) observed that the aquatic macrophyte *Hydrocotyle ranunculoides* L.f. presented high potential for bioremediation for several trace elements (mainly Iron and Aluminum) from the rizofiltration method.

Table 1. Advantages and disadvantages of using of the phytoremediation approaches to remove trace elements in aquatic environments.

Advantages	Disadvantages
Low maintenance cost and considered environmentally friendly	The concentrations of trace elements in the environment must be within the tolerance limits of the selected species
Easy acceptance of the general public	Some plants hyperaccumulate only a few trace elements
Contaminated plants are easy to remove from the environment	Biomagnification can occur from the plants used
Can be used <i>in situ</i> and <i>ex situ</i>	Care of the use of non-native species in contaminated environments

Adapted from Lee (2013), Favas et al. (2014) and Singht et al. (2012).

5. Final remarks

Increasing concentrations of trace elements in aquatic environments have been considered a relevant environmental pollution. Although most studies involving this type of pollution are associated with point sources of pollution, agricultural expansion and economic development is an eminent activity nowadays. However, the pressure that this activity generates on natural resources, especially water bodies, needs to be better understood. In this brief review, we sought to gather information about the main characteristics of trace elements, their effects on aquatic organisms and ways of remediation of these impacts. However, the effectiveness of the application of mitigation and remediation approaches and technologies will only be effective with (1) the formation of a solid conceptual basis regarding the effects that the trace elements can cause to the aquatic fauna and flora and human health; (2) a real interest of the public managers, in the different government spheres, to foment the improvement of these mitigating mechanisms and human resources qualification and, finally; (3) to have a clear dialogue between managers and science researchers, once that these two groups have a basic role in the process. On one side, the researchers with the knowledge and on the other side the managers with the possibility of action. However, a more participative society can collaborate with the effectiveness of this cooperation, supervising the actions that must be done with responsibility and commitment, for the conservation and recovery of natural resources (Figure

2).

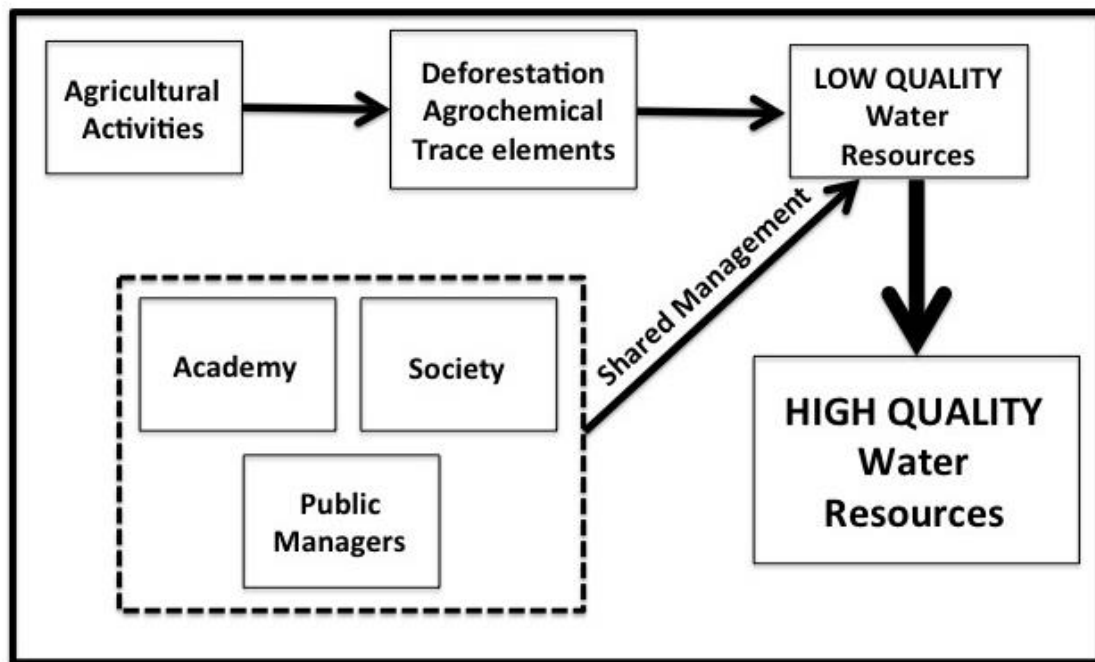


Figure 2. Schematic representation of shared actions among academia, managers and society in favor of improving the quality of water resources.

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CAPÍTULO 2

The influence of the environment in the incorporation of copper and cadmium in scraper insects



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The influence of the environment in the incorporation of copper and cadmium in scraper insects

Abstract

In regions with intense agricultural activity, increases in heavy metal concentrations in aquatic environments are common. Among the metals associated with agricultural activities, Copper (Cu) and Cadmium (Cd) have been found to directly affect aquatic trophic structures due to the ease of incorporation by primary producers and consuming organisms. Aquatic insects are predominantly found in streams, and their presence is determined by environmental characteristics and food availability. In this study, we seek to understand how the incorporation of Cu and Cd by scraper insects relates to their environment and food sources. We collected water, sediment, biofilm, and scraper insect samples in streams with different intensities of agricultural activities in the drainage areas. The intensity of agricultural activities in the catchment area positively influenced the Cu and Cd concentrations in organisms and other aquatic compartments. The metals were readily incorporated by the biofilms. Although the functional characteristics are important to understand the functioning of ecosystems, in this study, we found that the physiological characteristics can be determinants in the concentrations of metals in aquatic insects.

Key-words: land use, biofilm, heavy metals, Ephemeroptera, sediment, bioaccumulation.

Introduction

Anthropic activities have been altering the structure and functioning of natural ecosystems in virtually every region of the world (Foley et al. 2011). In this context, changes in land use stand out for having negative impacts on ecosystems and affecting ecosystem services, especially regulating and provisioning services (Allan 2004). Among the different land uses, agricultural activities are one of the main sources of surface water contamination (Loureiro et al. 2018; Molina et al. 2017; Yang et al. 2013). In Brazil lands, agrochemicals used in agricultural may contain metal in their formulations and, consequently, increase soil concentrations (Brito et al. 2020), and drain into water bodies, contaminating sediments and water (Silva et al. 2017). Due to their persistence and toxicity, increasing metal concentrations in aquatic ecosystems has been considered one of the main threats to aquatic fauna and flora (Aguilar-Hinojosa et al. 2016).

In Brazil, agricultural production has grown exponentially in recent years, with a great diversity of food produced. Concomitant to this growth, since the last decade Brazil has been the largest consumer of pesticides in the world (Fernandes et al. 2020). Thus, the increase in metal concentrations in streams influenced by agricultural activities has already been reported in different crops, such as sugarcane (Corbi et al. 2008), banana (Corbi et al. 2013) and soy (Loureiro et al. 2018).

Cadmium and copper are metals that have negative effects on aquatic communities (Hunting et al. 2013; Loureiro and Hepp 2020; Prommi and Payakka 2018). The use of phosphate fertilizers in agriculture is considered an important diffuse source of contamination by cadmium (Cd) in aquatic environments (Pan et al. 2010). Cd is a nonessential element that has a high potential for toxicity to organisms (Mebane 2010). Cd can be found in different compartments in aquatic ecosystems such as sediment, water, and biota (Prommi and Payakka 2018). In turn, copper (Cu) is essential to life and is involved in many enzymatic and metabolic processes (e.g., at least 30 enzymes functioning as redox catalysts) (Flemming and Trevours 1989). However, at higher concentrations, Cu can also be toxic to organisms, affecting the processes that they catalyse, leading to, for example, reduction in consumption rates of shredders insects and consequently affecting the decomposition process (Zubrod et al. 2015). Cu has been widely used in agriculture in fungicides, bactericides, and herbicides (Kwan et al. 2015; Zubrod et al. 2015).

Aquatic insects can be exposed to metals by different compartments (e.g., food, water or sediment) or a combination of different factors (Adams et al. 2011; Loureiro et al. 2018).

This exposure can increase the levels of metal concentrations in organisms. Aquatic insects become an important group for monitoring aquatic environments impacted by metals, since they can reflect the conditions of the environment (e.g., increased concentrations of metals in the sediment) (Santoro et al. 2009). In addition, metals that are easily incorporated into the base of the trophic chain (e.g., by biofilms; Hua et al. 2019) can be mobilised and contaminate higher levels of the trophic web (Croteau et al. 2010; Loureiro et al. 2018; Pastorino et al. 2020). Thus, the bioaccumulation of metals can eliminate sensitive species and consequently alter the structure and composition of aquatic insects (Hepp et al. 2017), and changes in interspecific relationships (Gardham et al. 2014).

Biofilms generally consist of communities of algae and bacteria that adhere to the surface of rocks and vegetation debris in rivers and streams (Ponsatí et al. 2016). Biofilms have a high capacity to accumulate heavy metals (Ancion et al. 2010). Biofilm-associated communities are considered one of the primary producers in streams (Franken et al. 2005) and can be considered an important contamination route for aquatic communities (Ancion et al. 2010; Loureiro et al. 2018).

In small streams, insects have complex trophic structures (Motta and Uieda 2004), and feeding strategies are characteristics that eventually reflect adaptations to environmental conditions (Tomanova et al. 2006). In tropical and subtropical regions, the main scraper insects belong to the order Ephemeroptera, particularly to the families Baetidae and Leptophlebiidae (Baptista et al. 2006). Ephemeroptera have been used as indicators of environmental quality in numerous studies (Clements et al. 2013; Rodriguez et al. 2018; Souto et al. 2019). Scrapers can bioaccumulate large amounts of metals, especially via biofilm consumption, subsequently transferring the elements to higher trophic levels (Rodriguez et al. 2018).

Considering the intensive agricultural practice with high use of agrochemicals in crop management, aquatic ecosystems are highly vulnerable to the impacts of these chemicals. In this sense, our study proposes to evaluate the direct and indirect effects of heavy metals on aquatic insects. The studied insects constitute intermediate levels of aquatic food chain. In addition, the possible negative effects of metals on these organisms can affect higher trophic levels, including people who depend on contaminated water resources. Therefore, we verified whether soil uses and the concentrations of Cu and Cd in the aquatic environment influence the concentrations of Cu and Cd in two species of scraper insects. For this, we tested the hypotheses: (i) that agricultural uses will be positively correlated with the incorporation of Cu and Cd by scrapers; (ii) that Cu and Cd concentrations in scrapers will be positively related to

metal concentrations in biofilms and water, and; (iii) that the sediment will not influence Cu and Cd concentrations in organisms, since the scrapers are not in direct contact with this freshwater compartment.

Material and methods

Study area

We conducted this study in the upper portion of the Uruguay River watershed in southern Brazil (27°45'S; 52°25'W) (Fig. 1; Table 1). The region has a temperate subtropical climate (Köppen type Cfb), with an average annual temperature of $17\pm1^{\circ}\text{C}$ and annual rainfall of 1900–2200 mm (Alvares et al. 2013). Vegetation is transitional between Araucaria and Seasonal Semideciduous forest as part of the Atlantic Forest Biome (Oliveira-Filho et al. 2015). The edaphic and geological formation of the region is basaltic, and the soil is predominantly Latosol (i.e., typical Alumino-Ferric Red Latosol, Lvaf type) (Streck et al. 2008).

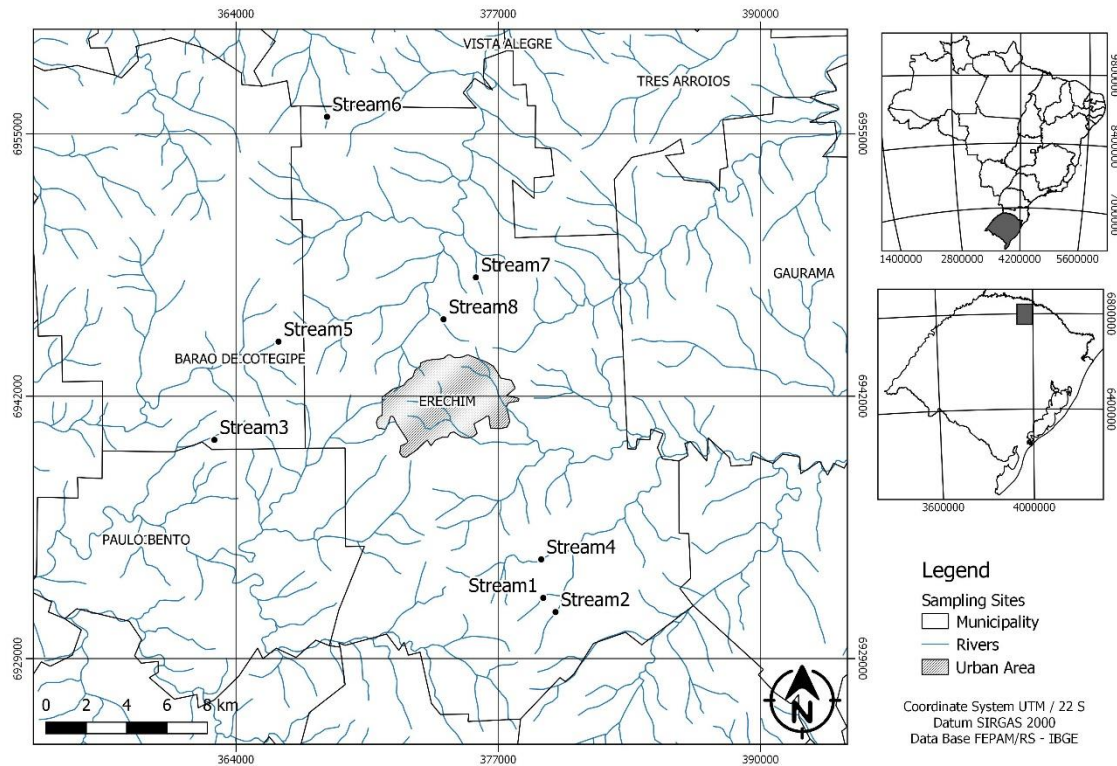


Fig. 1 Geographic location and distribution of streams studied in Southern Brazil.

As a choice of collection sites, we select streams $\leq 2^{\text{nd}}$ order, an average width of ~ 2 m and a depth of < 0.25 m during the study period, also, as a way of selecting the collection

points, we consider a gradient of agricultural activities in the drainage area of each location. Agricultural activities account for about 80% of the entire area of the region (Rovani et al. 2019), with soybeans being the main crop in the austral summer, and wheat and pastures in the austral winter (Fontaneli et al. 2000; Santos et al. 2014). Samples were collected between February and March 2018, so at the end of soybean cultivation. The Stream1, Stream2, Stream3 Stream4 sampling points are strongly influenced by agricultural activities, with >72% of agricultural activities in its drainage area (Table 1). The Stream5 and Stream6 sampling points are located in an agricultural region, however, with transition areas between agriculture and natural vegetation at drainage area. The Stream7 and Stream8 have a large area of natural vegetation.

Table 1. Geographic coordinates and percentage of land uses measured in streams studied in Southern Brazil.

Streams	Geographical coordinates	Natural vegetation (%)	Forestry (%)	Pasture (%)	Agriculture (%)
Stream1	27°73'08"S 52°21'82"W	5.9	0.5	5.3	88.0
Stream2	27°73'12"S 52°21'70"W	8.6	0.5	3.5	87.4
Stream3	27°64'95"S 52°39'78"W	18.9	2.4	5.7	73.0
Stream4	27°71'33"S 52°22'54"W	15.6	0.3	11.4	72.7
Stream5	27°61'46"S 52°32'77"W	31.8	2.8	6.6	58.8
Stream6	27°48'35"S 52°30'90"W	29.5	7.9	17.5	45.1
Stream7	27°58'63"S 52°25'73"W	43.8	0.0	31.8	24.4
Stream8	27°60'33"S 52°27'66"W	48.0	2.1	15.6	19.1

Land use analysis

For each stream, we calculated the drainage area following the cartographic parameters of selection of quoted points and water dividers using the software MapInfo Professional 8.5 and Idrisi Selva. We used the 1:50,000 scale cartographic base consisting of articulated leaves and Landsat-8 OLI/TIRS sensor images with a spatial resolution of 30 m. We quantified land use classes following the cartographic parameters of water body selection, and use of Boolean operations, distance, and context operations of GIS applications. Land use classification followed the Maxlike Supervised Classification method (maxlike) in GIS

IDRISI 32, based on field sampling units using GPS with prior knowledge of the area. For this study, we adopted the land use classes: (i) agriculture, (ii) natural tree vegetation, (iii) forestry, and (iv) pasture. We use these usage classes as they are prevalent in the study region (Rovani et al. 2019).

Field sampling

For each flow, we perform a single collection of the water ($n = 3$ per stream), sediment ($n = 3$ per stream), biofilm samples ($n = 3$ per stream), and insects in each stream. In each stream, we measured the total electrical conductivity, pH, dissolved oxygen, turbidity, water temperature, and dissolved solid with the multiparameter analyser HORIBA®. In addition, we collected approximately 500 mL of water from each stream to quantify dissolved organic carbon (DOC) and total dissolved nitrogen (TDN). In the laboratory, we filter the water samples with a 0.45 μm filter and perform the quantification of DOC and TDN by oxidation and combustion in a TOC-VCSH analyzer (SHIMADZU®). The analysis methodologies are described in Standard Methods (APHA, 1998). The sediment was sampled with a 70 mm diameter corer at <10 cm depth. After collection, the pellet was dried at $50^{\circ}\text{C}/72$ h and sieved using a 0.063 mm mesh sieve to quantify potentially bioavailable concentrations.

In each stream, we collected the biofilm by scraping the stony substrate and the samples were transported on ice to the laboratory for oven drying (50°C for 24 h). We collected aquatic insects in streams with a Surber sampler (250 μm mesh; 0.09 m^2 area) (minimum 3 per area) and visual searches in banks of leaves and stones in the bed of streams. The samples were transported on ice to the laboratory for identification (Fernández and Domínguez 2001; Costa et al. 2006). As model organisms, we selected nymphs of the genera *Baetodes* (Baetidae, Ephemeroptera) and *Hagenulopsis* (Leptophlebiidae, Ephemeroptera). These genera are classified as scrapers (Milesi et al. 2016; Graça et al. 2018) and have a wide distribution and abundance in streams in southern Brazil, particularly in the studied watershed (Milesi et al. 2016). In this study, we collected 61 *Baetodes* nymphs (body size: 5.3-6.4 mm) and 58 *Hagenulopsis* nymphs (body size: 4.8-5.9 mm). *Baetodes* were present in seven streams (Stream1, Stream2, Stream3, Stream4, Stream5, Stream6 and Stream8), while *Hagenulopsis* were found in five streams (Stream1, Stream2, Stream4, Stream6 and Stream7). For each stream, we analyzed a minimum of three samples, composed of a pool of individuals

(3-5 larvae). The larvae pool per sample was used to obtain a minimum biomass for extraction and analysis of metals.

Metals analysis

We determined Cu and Cd concentrations in the water, sediment, biofilm samples, and aquatic insects. For this, we extracted the sediment metal fraction from ~0.5 g sediment (fraction <0.063 mm), and we add 10 mL 0.1 mol L⁻¹ HCl for metals extraction. Subsequently, the samples were filtered (PVDF filter, 0.45 µm diameter) in a 25 mL volumetric flask and the total volume was measured with 0.1 mol L⁻¹ HCl. For the water samples, we filtered ~20 mL of the collected water and added 3 mL of HNO₃. We dried the biofilm samples and insects in a greenhouse (50°C for 72 h) and weighed portions of 0.3–0.5 g for acid digestion. We extracted insect metals from fractions of 1–100 mg dry weight. However, due to low nymph biomass, we grouped 2–4 nymphs per sample for acid digestion. Samples were digested with a solution of 4 mL of HNO₃ and 1 mL of H₂O₂ at room temperature (~25°C for 5–7 days) (Rodriguez et al. 2018). Subsequently, the insect and biofilm samples were filtered and placed in a 10 mL volumetric flask, and the total volume was measured with 1 mol L⁻¹ HNO₃. We analysed Cu and Cd concentrations by graphite furnace atomic absorption spectrophotometry (AAnalyst AAS-Perkin Elmer® Mod 800). Calibration curves were prepared by serial dilutions of the stock solution (1000 mg L⁻¹; standard SPEX CertiPrep®). The detection limits were 0.13 and 0.10 µg L⁻¹ for Cu and Cd, respectively, and the quantification limits were 0.43 and 0.30 µg L⁻¹ for Cu and Cd, respectively.

Data analysis

Initially, we transformed the values of the matrix of physical and chemical water variables into log (x+10), but for the land use matrix, we used an arcsine transformation. We synthesised the average variation in the physical and chemical variables of water and land use, independently, with a Principal Component Analysis (PCA). We used this criterion, as we understand that physical and chemical variables and land uses matrices reflect different scales (i.e., stream and drainage area, respectively). In this way, we synthesise the variability generated and use the scores of principal components (PC) as environmental predictors on both scales. In the two PCA, we verified the number of significant PC for interpretation from

a Broken Stick analysis (Jackson, 1993). We used this criterion to select the PC that would be used as explanatory variables in the regression models.

Multiple linear regressions were used to evaluate how the Cu and Cd concentrations of the scraper insects were related to environmental conditions (i.e. physical and chemical variables of water and land uses) and metals found within biofilms, sediments, and water. For each response variable (i.e., Cu and Cd in both insect genera) we sought the appropriate minimum model through manual model selection in which non-significant explanatory variables ($p > 0.05$) were removed from the analysis until only significant variables remained ($p < 0.05$). For this, we tested the multicollinearity of the explanatory variables from the variance inflation value (VIF), considering only those variables with values <10 in the appropriate minimum models used (Quinn and Keough 2002). The variables that are removed due to multicollinearity will be subjected to a Pearson correlation test with the significant explanatory variables of the multiple regression model. We performed Pearson linear correlation analyses to verify the relationship between environmental conditions (i.e. physical and chemical water variables and land uses) and Cu and Cd concentrations of the biofilm, sediments, and water column on the metals concentrations. For the application of VIF, we used the *car* package (Fox and Weisberg 2011). For the accomplishment of PCA and Broken Stick we used the *vegan* package (Oksanen et al. 2017). For all statistical analyses we used R version 3.6.1 (R Core Team, 2017).

Results

Turbidity, TDN, and DOC were physical and chemical variables that presented the greatest variation among streams (Table 2). Turbidity ranged from 0.4–4.7 NTU, while TDN concentrations ranged from 1.4–4.0 mg L⁻¹ and DOC from 3.3–17.8 mg L⁻¹ (Table 2). Agriculture and natural tree vegetation were the predominant land usages and land cover classes in the area surrounding the streams (Table 2). The percentage of agricultural land use in stream drainage areas ranged from 19–88%, while the percentage of natural tree vegetation ranged from 5–48% (Table 2).

Water and sediment Cu concentrations ranged from 1.4–24.9 µg L⁻¹ and 4.8–230.8 mg kg⁻¹, respectively (Table 3). Cd concentrations in water ranged from 0.5–1.8 µg L⁻¹, whereas in the sediment this element ranged from 2.4–7.3 mg kg⁻¹ (Table 3). On the other hand, Cu concentrations in the biofilm samples ranged from 37.2–671.1 mg kg⁻¹ and Cd ranged from 26.7–325.3 mg kg⁻¹ (Table 3).

Table 2. Physical and chemical variables of water measured in streams studied in Southern Brazil. DO = dissolved oxygen; TDS = total dissolved solids; TDN = total dissolved nitrogen; DOC = dissolved organic carbon.

Streams	Temperature (°C)	pH	Electric conductivity ($\mu\text{S cm}^{-1}$)	Turbidity (NTU)	DO (mg L^{-1})	TDS (mg g^{-1})	TDN (mg L^{-1})	DOC (mg L^{-1})
Stream1	8.4	7.7	130	2.6	13.8	0.085	4.0	4.8
Stream2	9.9	7.8	132	0.9	11.5	0.086	3.3	17.8
Stream3	14.0	8.0	111	2.3	15.0	0.072	2.4	3.3
Stream4	9.8	7.7	93	0.4	13.1	0.061	3.2	11.7
Stream5	12.7	7.3	91	4.7	16.8	0.059	4.1	6.2
Stream6	8.0	7.8	97	2.2	14.0	0.063	2.7	5.3
Stream7	9.5	7.7	88	4.4	12.1	0.057	1.4	4.6
Stream8	10.0	7.9	78	1.0	11.4	0.051	3.0	10.2

Table 3. Copper and Cadmium concentrations in water, sediment and biofilm (mean $\pm$ standard error) in streams studied in Southern Brazil

Streams	Copper			Cadmium		
	Water ($\mu\text{g L}^{-1}$)	Sediment (mg kg^{-1})	Biofilm (mg kg^{-1})	Water ($\mu\text{g L}^{-1}$)	Sediment (mg kg^{-1})	Biofilm (mg kg^{-1})
Stream1	2.8 $\pm$ 0.0	207.0 $\pm$ 12.1	637.8 $\pm$ 166.6	1.2 $\pm$ 0.1	7.2 $\pm$ 0.0	291.4 $\pm$ 328.1
Stream2	17.3 $\pm$ 3.9	192.2 $\pm$ 2.5	249.7 $\pm$ 243.5	0.9 $\pm$ 0.2	4.3 $\pm$ 0.2	222.9 $\pm$ 8.7
Stream3	1.6 $\pm$ 0.2	42.5 $\pm$ 30.9	185.0 $\pm$ 488.2	1.1 $\pm$ 0.1	5.5 $\pm$ 0.0	125.3 $\pm$ 8.8
Stream4	3.7 $\pm$ 0.4	124.4 $\pm$ 5.7	123.9 $\pm$ 17.9	0.8 $\pm$ 0.1	5.1 $\pm$ 0.1	157.0 $\pm$ 35.7
Stream5	10.3 $\pm$ 0.4	28.6 $\pm$ 0.2	59.7 $\pm$ 148.6	0.9 $\pm$ 0.0	3.0 $\pm$ 0.0	39.0 $\pm$ 4.8
Stream6	1.6 $\pm$ 0.0	47.5 $\pm$ 3.7	35.9 $\pm$ 27.3	0.9 $\pm$ 0.1	3.6 $\pm$ 0.1	64.9 $\pm$ 18.6
Stream7	10.4 $\pm$ 0.0	30.2 $\pm$ 0.6	248.6 $\pm$ 231.8	1.1 $\pm$ 0.2	3.0 $\pm$ 0.3	39.7 $\pm$ 8.3
Stream8	1.7 $\pm$ 0.0	4.8 $\pm$ 0.0	70.0 $\pm$ 142.3	1.6 $\pm$ 0.0	2.7 $\pm$ 0.1	27.9 $\pm$ 7.4

Cu concentrations in *Hagenulopsis* ranged from 39.7–273.2 $\mu\text{g g}^{-1}$, while *Baetodes* ranged from 27.6–213.4 $\mu\text{g g}^{-1}$ (Fig. 2A). The incorporation of Cd by *Hagenulopsis* ranged from 0.4–4.2 $\mu\text{g g}^{-1}$, and by *Baetodes* ranged from 0.2–1.1 $\mu\text{g g}^{-1}$ (Fig. 2B).

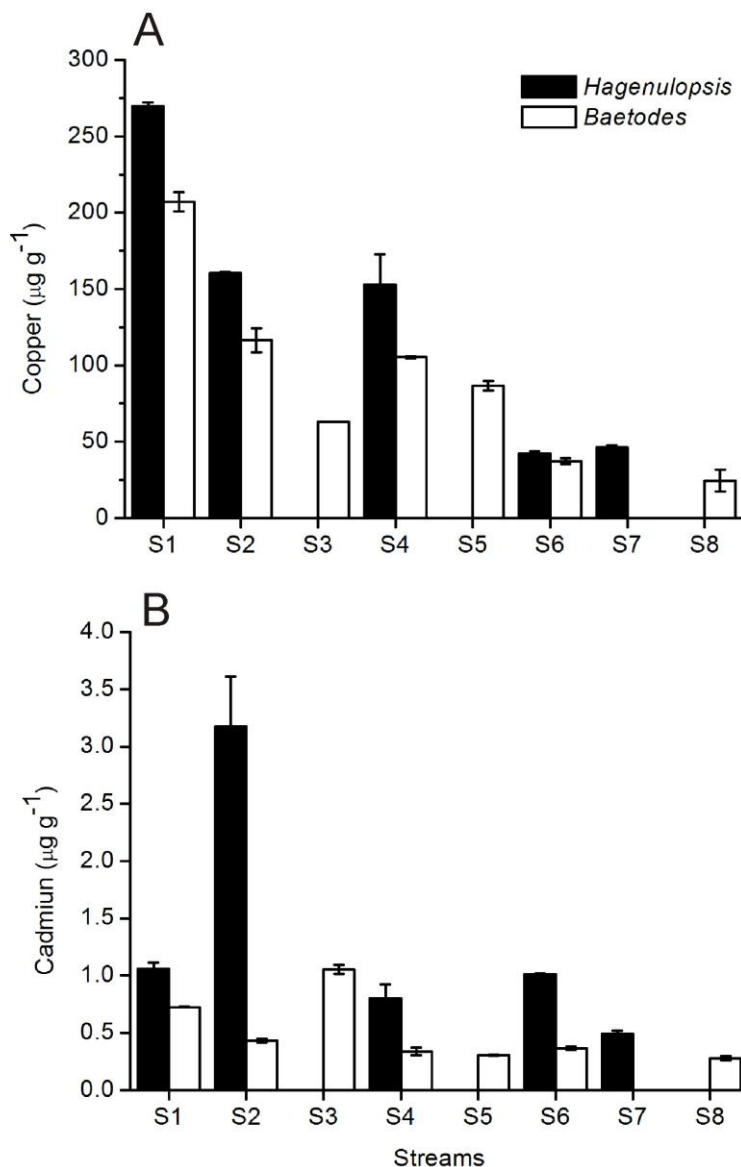


Fig. 2 Mean concentrations $\pm$ standard error of Copper (A) and Cadmium (B) for *Baetodes* and *Hagenulopsis* in the eighth streams studied in southern Brazil.

Only the first PC of each PCA was significant in each of the performed PCA. For the physical and chemical variables of water, PC1 explained 69.4% of total data variation, being positively related to turbidity and negatively to DOC (Fig. 3). The PC1 of the ordering performed with land use data explained 90.1% of the data variation, with agricultural land use found to be positively related to this component while natural tree vegetation was negatively related (Fig. 4).

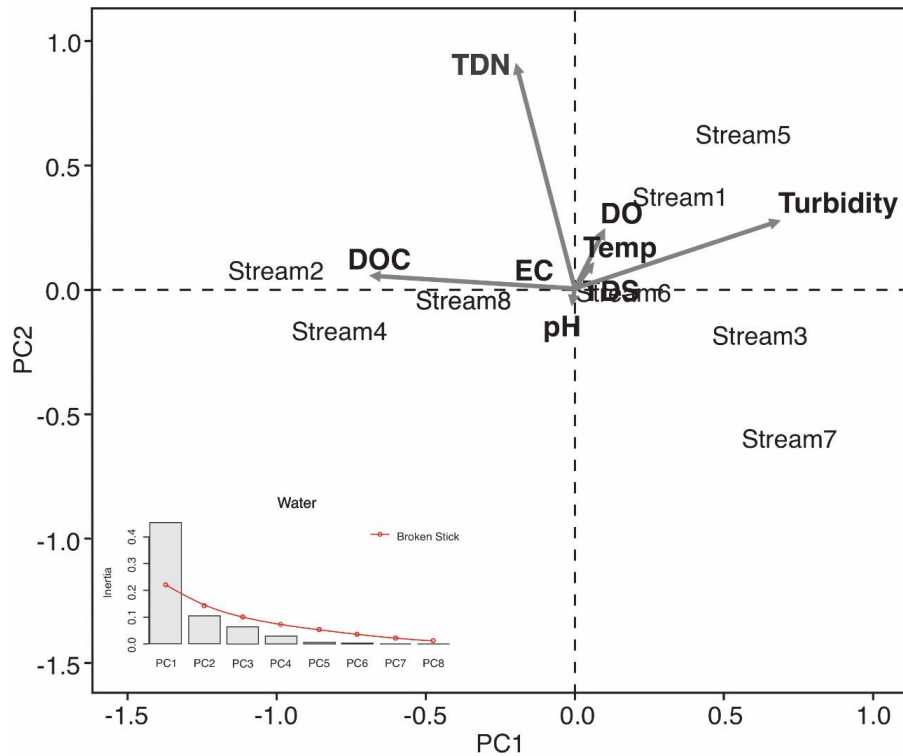


Fig 3. Principal component analysis and Broken Stick of the physical and chemical variables of water in streams studied in Southern Brazil. The first principal component (PC1) explained 69.4% of the data variation. DOC = dissolved organic carbon; DO = dissolved oxygen; TDN = total dissolved nitrogen; Temp = temperature; TDS = total dissolved solid.

When performing multicollinearity tests, we removed the variable Cu concentration in the sediment for the Cu concentration model in *Hagenulopsis*, and we removed the PC1 land uses from the Cd concentration models in *Hagenulopsis* and *Baetodes*. The effects of explanatory variables on Cu and Cd concentrations incorporated by scrapers varied among organisms. Cu concentrations in *Baetodes* were explained by the land uses (agriculture) (Table 4). In *Hagenulopsis*, Cu concentrations were explained by the concentration of Cu in biofilms and land use (agriculture) (Table 4). Cd concentrations in *Baetodes* were explained by the concentrations of this element in sediments and biofilms, and the level of turbidity. For *Hagenulopsis*, Cd concentrations were explained by the concentration of this element in sediments, and by the land use (Table 4).

The Cu in sediment from the Cu regression model in *Hagenulopsis* was positively correlated with the landscape PC1 ($r = 0.98$, $p < 0.01$) and with the biofilm ($r = 0.50$, $p = 0.01$). For Cd regression model in *Hagenulopsis* the variable PC1 land uses showed a positive correlation with sediment ($r = 0.70$, $p < 0.01$), with biofilm ($r = 0.93$, $p < 0.01$) and with PC1 water ($r = 0.82$, $p < 0.01$). For Cu regression model in *Baetodes*, the PC1 land uses showed a positive correlation with the sediment ($r = 0.68$, $p < 0.01$) and for biofilm ($r = 0.98$, $p < 0.01$).

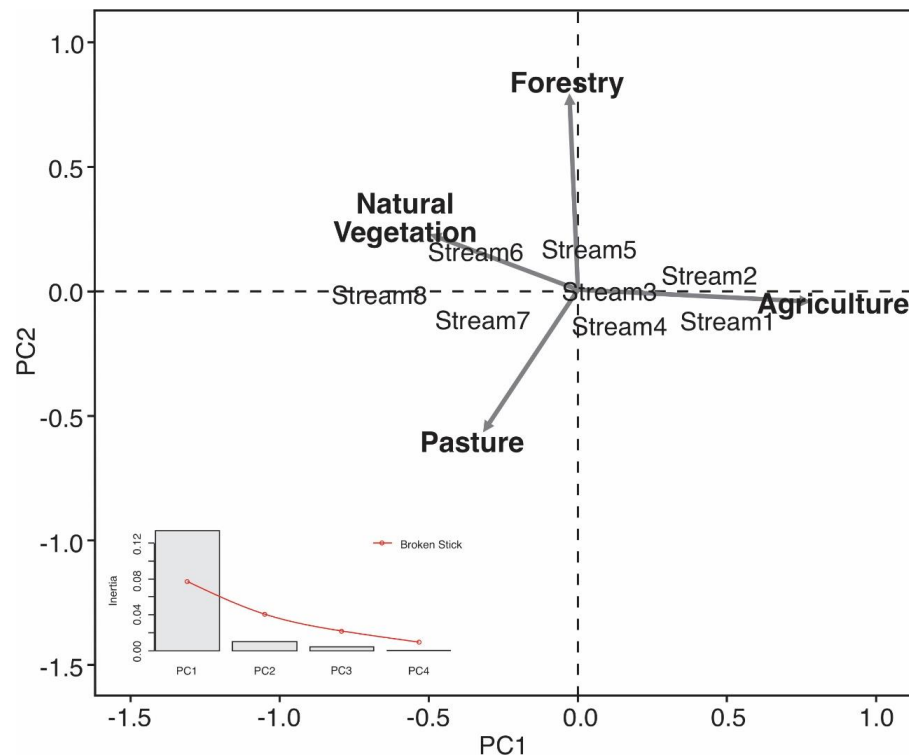


Fig 4. Principal component analysis and Broken Stick of the percentage of land use in the drainage area of the study region. The first principal component (PC1) explained 90.1% of the data variation.

Table 4. Analysis of the minimum models Copper and Cadmium on *Baetodes* and *Hagenulopsis* insects sampled in streams studied in Southern Brazil.

	Estimate	Standard error	T	p	R ² adjusted	df
Copper						
<i>Baetodes</i>						
Intercept	-33.65	4.20	0.98	<0.01	0.77	22
PC1 _{land use}	16.49	1.81	4.04	<0.01		
<i>Hagenulopsis</i>						
Intercept	-28.72	4.10	-6.99	<0.01	0.84	18
Biofilm	0.22	0.07	2.91	<0.01		
PC1 _{land use}	13.99	1.84	7.58	<0.01		
Cadmium						
<i>Baetodes</i>						
Intercept	0.69	0.30	2.27	0.03	0.67	20
Sediment	0.26	0.04	5.36	<0.01		
Biofilm	-0.02	0.00	-3.55	<0.01		
PC1 _{water}	0.46	0.12	3.66	<0.01		
<i>Hagenulopsis</i>						
Intercept	4.44	0.51	8.58	<0.01	0.62	18
Sediment	-1.11	0.23	-4.75	<0.01		
Biofilm	0.19	0.03	5.95	<0.01		

Discussion

The Cu and Cd concentration by studied insect genera varied according to stream characteristics and organisms. Both organisms mainly feed biofilm (Graça et al. 2018), however, differences in the incorporation of metals between the genera may be associated to the way that these animals feed. *Hagenulopsis* uses bristles to remove lightly deposited material in the biofilm (Baptista et al. 2006), while *Baetodes* has structural adaptations that allow them to reach material that is firmly fixed onto the substrate. As the biofilm consists of a set of microorganisms (e.g., algae, bacteria, fungi) that have different morphologies and ways of fixing to the substrate (Lin et al. 2019), the two insect genera may be consuming different groups and amounts of the resource, and consequently accumulating different metals amounts. In addition, differences between species in the same environment may be associated with the ability to store or eliminate the metal it absorbs (Hare 1992).

In environment, the feeding mayflies seems to be an important source of exposure for Cu and Cd (Cain et al. 2011). The variability between Cu and Cd concentrations in organisms may be being driven by physiological (e.g. storage and detoxification) and non-functional factors (e.g., form and type of food) (Buchwalter et al. 2008). Although important for several types of ecological studies, the functional approach must be carried out with care, since the physiological characteristics of each species can affect the metal concentrations of organisms. Higher metal concentrations were associated with biofilms, concentrations within this compartment did not necessarily exert a direct influence on the Cu and Cd concentrations incorporated by the scrapers. The contributions of metals from different exposure sources are associated with the bioavailability of metals in different compartments (Rainbow 2002). In addition, the accumulation of metals in organisms may also depend on whether the metal will be detoxified/excreted or available to play a role in metabolism (Luoma and Rainbow 2005).

Our first hypotheses was corroborated in all models, where agricultural activities influenced the Cu and Cd concentrations in the studied organisms. Our results indicate a positive correlation between agricultural intensity in stream drainage area and Cu and Cd concentrations in organisms. The studied region has a predominantly agricultural matrix, producing on a large scale some monocultures, such as corn, wheat and mainly soy (Rovani et al. 2019). The development of agricultural activities associated with public measures that do not control the use of pesticides and fertilizers in crops, make aquatic ecosystems sources sensitive to increased concentrations of metals and other chemical compounds. The presence of metals in agriculturally-influenced streams is due to contaminated soil entering the aquatic

environment (Jergentz et al. 2005; Nystrand et al. 2012). The greater availability of metals in streams makes these elements easily incorporated by aquatic organisms (Loureiro et al. 2018). These suspended solids are small particles of the sediment itself, which decant and settle on biofilms, facilitating the consumption of metals by scrapers (Latrille et al. 2019).

On the other hand, our second hypothesis was partially corroborated. The Cu and Cd concentrations dissolved in water were not significant variables on Cu and Cd concentrations in scraper insects, thus refuting our hypothesis. The metal dissolved fraction is the most mobile and bioavailable, generally being the most toxic fraction (Nystrand et al. 2012). However, the Cu and Cd concentrations did not show great variation between the streams studied. Metal ions remain for a short time in the water column (Soares et al. 1999), generally reacting and accumulating in the sediment (Magalhães et al. 2015). In addition, the increase in turbidity reflects the amount of suspended solids in the water column. The biofilm, showed to be an important compartment in relation to the Cu and Cd concentrations in scraper insects. In this study, Cu and Cd concentrations in the biofilm were higher than other compartments studied (i.e. sediment). These results corroborate other studies into Cu and Cd, as well as previous studies into different metals and metalloids such as arsenic and zinc (Ancion et al. 2013; Hepp et al. 2017; Loureiro et al. 2018). As biofilms are considered large reservoirs of organic matter that can accumulate metals (Croteau et al. 2005), in regions with agricultural activities around streams, biofilm can be an indicator of metal contamination by (i) the removal of vegetation cover, favoring primary productivity by increasing luminosity and favoring proliferation of biofilm biomass (Franken et al. 2005; Tonin et al. 2018) (ii) are an important source of resources for a good part of the fauna of primary consumers in streams (Graça et al. 2018).

The positive correlation between sediment and agricultural activities strengthens the evidence that this limnic compartment is an important metal detection source in streams. The particle size structure of the sediment affects its ability to retain chemical elements (Nystrand et al. 2016). When associated with biota, only a small fraction is in bioavailable forms, the largest fraction of metals is immobilized in sediments in non-bioavailable forms (Nystrand et al. 2012), nevertheless, changes in redox potential may increase the bioavailability of metals (Zoumis et al. 2001). In this way, many metals accumulate in the sediment and are only released into the water column when there is a change in the physical and chemical characteristics of the water (e.g. pH decrease) (Wang et al. 2016). In addition to having a high potential for metal accumulation, the sediment can be a serious contamination problem over time (Božanić et al. 2019; Gabriel et al. 2020). Thus, our results indicate that metal

concentrations in sediment are an important source of contamination for aquatic scraper insect.

Conclusion

In this study, we seek to understand the variation of copper and cadmium concentrations in scrapers insect from food sources and environmental characteristics. We found that environment and metal concentrations relationships in aquatic organisms vary between the studied heavy metal and Ephemeroptera genera. Combinations of taxonomy with functional characteristics of organisms may be important in determining the relationships between metal concentrations in the environment and aquatic fauna. Initially, we found that the agricultural activities intensity in streams drainage area influences the Cu and Cd concentrations in scraper insects. Although we have studied only one trophic group (i.e., scraper insects), our results show that the metal contamination of basal resources (e.g., biofilms) in aquatic environments is very high. This facilitates the incorporation of metals into the trophic chain and, depending on the characteristics of the metal, can be biomagnified to higher trophic levels. .

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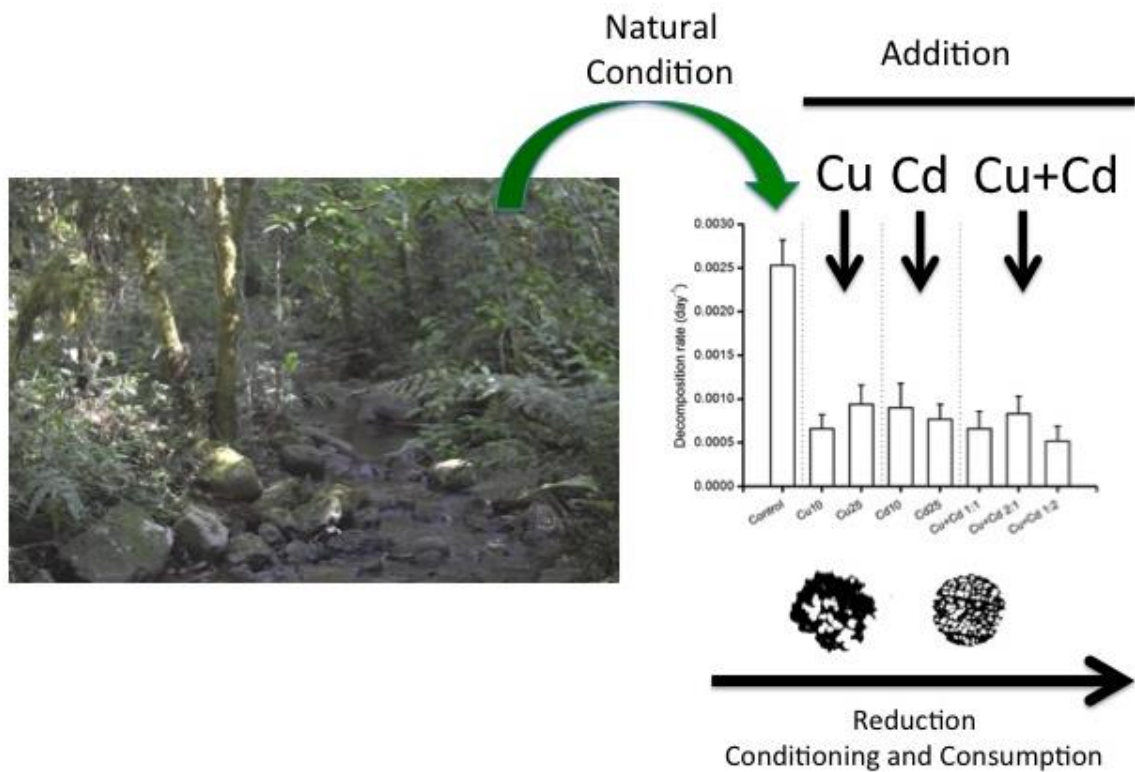
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CAPÍTULO 3

Effects of copper and cadmium on stream leaf decomposition: evidence from a microcosm study



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Effects of copper and cadmium on stream leaf decomposition: evidence from a microcosm study

Abstract

We seek to understand how heavy metals (copper and cadmium) act on the decomposition of leaf litter by their effects on microbial conditioning and litter fragmentation by invertebrates. Under controlled conditions, we evaluated the microbial conditioning (sporulation rates as a proxy) and the consumption rates of *Nectandra megapotamica* detritus by *Phylloicus* sp. under different concentrations of copper and cadmium (alone and combined). We performed a microcosm with concentrations 10× and 25× higher than those in the natural environment for the two metals and their combination, evaluating their effects on microbial conditioning, the consumption rate by shredders and, consequently, the leaf litter decomposition rates. Sporulation rates was affected by all copper concentrations tested but significantly reduced only at the highest concentration of cadmium. Increased copper and cadmium concentrations reduced the consumption of leaf litter by *Phylloicus* sp. at 60%. As a consequence, we observed a decrease of ~3× in the decomposition rates in with metal concentrations 10× and 25×. When combined, copper and cadmium negatively affected microbial conditioning, consumption by shredders and leaf litter decomposition. Increases in concentrations of copper and cadmium directly affected organic matter decomposition in aquatic environments. Thus, the presence of high concentration of heavy metals in aquatic environments alters the functioning of ecosystems. As metals occur in a combined manner in environments, our results show that the combined effects of different metals potentiate the negative effects on ecosystem processes.

Keywords: Heavy metals, ecosystems functioning, conidia production, shredders

Introduction

Agricultural, industrial and mining activities are positively correlated with increased concentrations of metals in the environment (Kumar et al., 2019). Aquatic ecosystems are particularly vulnerable to the presence of metals as they become the main sources of their disposal from human activities, either by diffuse or non-diffuse sources (Loureiro & Hepp, 2020). The degradation of the environmental quality of aquatic ecosystems has caused several threats to biodiversity and human health (Dudgeon et al., 2006; Francisco et al., 2019). Metals are generally present in aquatic environments, having different relationships with aquatic biota (Stankovic et al., 2014), while essential metals are directly related to biological processes in living beings, non-essential metals do not have clear biological functions in organisms (Rainbow, 2002; Duarte et al., 2004). However, metals, even if essential, can be toxic at high concentrations, depending on the organism, the physical and chemical properties of the metal and the environmental conditions (Rainbow, 2002; Duarte et al., 2004).

Metals such as Copper (Cu) and Cadmium (Cd) have been gaining wide interest in the scientific community due to their harmful potential for aquatic organisms and human health (e.g., Tagliaferro et al., 2018; Loureiro et al., 2018; Menegat et al., 2020; Loureiro & Hepp, 2020; Mebane et al., 2020; Hadjipanagiotou et al., 2020). Cd is a non-essential element with a high toxic potential (Croteau et al., 2005). In natural environments, igneous rocks are responsible for high Cd concentrations in the environment (Mar & Okazaki, 2012). However, the use of phosphate fertilisers, atmospheric deposition and industrial and urban waste are responsible for the Cd contamination of ground and surface water (Ulrich, 2019). In some cases, Cd can replace essential elements and interfere with the enzymatic functioning of organisms (Torres et al., 2008). In contrast, Cu is an essential element in the life cycle of organisms (Rainbow, 2002). However, anthropic release (e.g., via fungicides and herbicides), associated with natural sources, contributes considerably to increasing Cu concentrations in aquatic systems (Kiaune & Singhasemanon, 2011). At high concentrations, Cu accumulates in the sediment and can be released into the water column (Segura et al., 2006). However, despite being an essential metal, Cu, when accumulated in excessive amounts, can have negative effects on aquatic biota (Handy, 2003).

The increase in the concentration of metals in aquatic environments has caused changes in terms of populations (Bergmann & Graça, 2020), communities (Duarte et al., 2008) and ecosystems (Duarte et al., 2004; Qu et al., 2010). At the ecosystem level, leaf decomposition is an important ecological process responsible for sustaining several biogeochemical cycles

(Graça, 2001). In streams, the decomposition process involves different biological interactions in the transformation of organic matter (Graça, 2001). Among the organisms involved in this process, aquatic fungi (i.e., hyphomycetes) are responsible for conditioning the detritus, aiding in the degradation of polysaccharides and increasing palatability for shredders (Arsuffi & Suberkropp, 1984). Shredders are responsible for the transformation of coarse particulate organic matter into fine particulate organic matter (Graça et al., 2015). However, several environmental factors influence the action of these organisms during the stages of processing organic matter, such as the structure of the riparian vegetation, the availability of nutrients or the concentration of metals (Graça, 2001; Ferreira et al., 2016).

Contamination by metals affects the organisms responsible for leaf decomposition, causing morphological deformities, reduced growth, changes in food intake and altered trophic interactions (Ferreira et al., 2016; Tagliaferro et al., 2018; Hepp et al., 2017; Deliberalli et al., 2018). More specifically, for Cd and Cu, several studies have reported negative effects of these metals on aquatic insect communities. Batista et al. (2012) found that the exposure of Cd ($\leq 35 \text{ mg L}^{-1}$) in shredder insects of the species *Limnephilus* sp. significantly affected leaf consumption. For Cu, Zubrod et al. (2014) found that the diet is an important source of contamination for shredders, where individuals of the species *Gammarus fossarum* exposed to Cu had their growth and lipid levels affected (reductions by 30 and 20%, respectively).

In the case of microbial decomposers, in contaminated environments, the number of species and reproductive activity of aquatic hyphomycetes can be considerably affected in these environments (Krauss et al., 2005), although the magnitude of these effects varies according to the metal type and concentration (Ferreira et al., 2016). Rousell et al. (2008) did not find significant differences between the rates of sporulation and alterations in the aquatic hyphomycete communities in a Cu-contaminated microcosm. On the other hand, Batista et al. (2012) found that the increase in Cd concentrations inhibited 50% of sporulation and affected the diversity of aquatic hyphomycetes. The production and dispersion of conidia are essential for the maintenance of species within a stream (Bärlocher, 2009). The decrease in the reproductive rates of hyphomycetes can generate direct effects, such as changes in microbial and indirect communities, or the reduction of leaf palatability for shredders (Biasi et al., 2019). The negative effects associated with adjacent processes in leaf decomposition suggest that high concentrations of metals can affect biological fragmentation (Ferreira et al., 2016). In this way, the action of shredders in metal-contaminated environments can alter organic matter decomposition in streams (Schaller et al., 2011).

In this study, we evaluated the effects of the metals Cu and Cd on microbial conditioning and on the consumption of leaf detritus by shredders under controlled conditions, with the aim to understand how Cu (essential metal) and Cd (non-essential metal) act on the decomposition of leaf detritus based on their effects on microbial conditioning and fragmentation by invertebrates. We tested the hypotheses that (i) only Cd, as a non-essential metal, will inhibit microbial conditioning, (ii) Cd and the highest concentrations of Cu will decrease the rate of leaf consumption by the shredders, and, because of this, (iii) the decomposition rate will be inhibited at higher concentrations of Cu and in the presence of Cd. We also tested the hypothesis that (iv) the mixture of the two metals will affect the microbial conditioning, rate of consumption by the larvae and decomposition of detritus in any combination of metals. This was expected because the mixtures of metals will enhance the adverse effects of these elements on organic matter decomposition.

Material and methods

This study was conducted under controlled conditions in a microcosm, considering three sequential steps: (i) preparation of microbial inoculum; (ii) conditioning by hyphomycetes and (iii) consumption of the detritus conditioned by shredders. Conditioning and consumption steps occurred in microcosms contaminated with Cu and Cd.

Plant detritus and metal treatments

Organic matter detritus, microbial inoculants and shredders invertebrates were collected in a stream (2nd order; 27°48'35"S and 52°30'09"W) in southern Brazil in the riparian zone of the Atlantic Forest, in a transition area between the Araucaria and Estacional Semidecidua forests (Oliveira-Filho et al., 2014). The main plant species in the riparian zone were *Nectandra megapotamica* (Spreng.) Mez, *Ocotea puberula* (Rich.) Nees, *Allophylus puberulus* (Cambess.) Radlk. and *A. edulis* (A.St.-Hil., Cambess. & A. Juss.) Radlk. (Capellesso et al. 2018). The stream presented an average width of ~3 m and a depth of < 0.25 m during the study period. The water was well oxygenated ($> 8 \text{ mg L}^{-1}$), basically pH neutral (~7), with a low electrical conductivity ($< 0.1 \text{ mS cm}^{-1}$) and a low nutrient concentration (phosphorus: $\sim 0.165 \text{ mg L}^{-1}$; nitrate: $\sim 2.3 \text{ mg L}^{-1}$).

We established eight treatments with different concentrations of Cu, Cd and Cu + Cd (mixture) and determined the basal concentrations of Cu and Cd from the average

concentrations observed in the stream water ($\text{Cu} = \sim 1.6 \mu\text{g L}^{-1}$ and $\text{Cd} = 0.9 \mu\text{g L}^{-1}$). We filtered 20 mL of the collected water samples and added 3 mL of HNO_3 ; Cu and Cd concentrations were analysed by graphite furnace atomic absorption spectrophotometry (AAAnalyst AAS-Perkin Elmer® Mod 800). Based on these concentrations, we defined the control treatments only with stream water, Cu10 and Cd10, with a 10× increase in the initial concentration of the respective metals, Cu25 and Cd25, with a 25× increase in the initial concentration, Cu + Cd 1:1, containing a mixture of both metals with concentrations 10× greater than the stream, Cu + Cd 2:1, containing a mixture of 25× more Cu and 10× more Cd and Cu + Cd 1:2, containing a mixture of 10× more Cu and 25× plus Cd. For the preparation of Cu and Cd solutions, we used standardised samples of $1,000 \text{ mg L}^{-1}$ (Merck 1.09987.0001 and Merck 1.09960.0001, respectively).

Preparation of the inoculum

To prepare the inoculum, we collected a volume of ~20 L of organic matter composed of a mixture of leaves of different plant species and different decomposition times, deposited in the benthic region of the stream. We collected about 15 L of water from the stream and transported all the material under refrigeration ($< 8^\circ\text{C}$) to the laboratory, where we deposited organic material and water from the stream in a glass container (30 L). The material remained at room temperature (20°C) for approximately 48 hours to enable leaching of soluble compounds, with oxygen saturation always above 90%. After this period, we divided the inoculum into eight treatments, each with approximately 400 g of wet detritus and 2 L of stream water, which constituted the different microcosms ($20 \times 15 \times 10 \text{ cm}$).

Microbial conditioning

We collected senescent and freshly fallen leaves of *N. megapotamica* (Spreng.) Mez on the floor of a single area before the start of the experiment. We chose this species because it has a wide distribution in the Atlantic Forest (Oliveira-Filho et al. 2014) and is commonly used in decomposition experiments (Tonin et al. 2018; Biasi et al. 2019). The leaves were dried at room temperature for 7 days, and subsequently, we cut 1,200 leaf discs (12 mm in diameter), avoiding the primary and secondary veins, and oven-dried them ($40^\circ\text{C}/48 \text{ h}$). We then placed sets of 30 leaf discs in litter bags ($6 \times 10 \text{ cm}$, 0.5-mm mesh); each treatment (described above) received five litter bags, which remained for 18 days for microbial conditioning. We

maintained the microcosms at controlled temperature ($18 \pm 1^\circ\text{C}$) and 12/12-hour photoperiod (light/dark), oxygen saturation $> 90\%$ ($\sim 8 \text{ mg L}^{-1}$). After this period, the litter bags were removed from the microcosms to quantify the rates of sporulation of the fungi ($n = 10$ discs) and the consumption rates of the shredders ($n = 20$ discs).

After conditioning the leaf discs, we stimulated the sporulation rate by incubating them in 250-mL Erlenmeyer flasks with 30 mL of the sampled water in an orbital shaker (100 rpm) for $18^\circ\text{C}/48 \text{ h}$. The suspension was filtered through a filter (25 mm, 5- μm pore size) and the conidia were stained (0.1% Trypan blue in 60% lactic acid), identified and quantified under an optical microscope ($400\times$) (Gulis et al., 2020). The discs were dried for $40^\circ\text{C}/48 \text{ h}$, and the sporulation rate, used as a proxy for the reproductive activity of the fungi, was expressed as conidia per mg of dry mass per time.

Litter consumption

As a typical shredder, we used larvae of the genus *Phylloicus* sp. (Calamoceratidae, Trichoptera). This organism is the most common shredder in the streams of the study region ($> 90\%$), with an important contribution to the processing of organic matter (Tonello et al. 2016, Tonin et al. 2018). We collected ~ 80 *Phylloicus* sp. larvae from the leaf banks distributed in the stream bed. The larvae were taken to the laboratory and kept in an aquarium with water from the stream for 48 h for acclimatisation (conditions similar to those for microbial conditioning). After this period, 65 larvae (sizes between 8 and 16 mm) remained alive and were used to assess the rate of leaf disc consumption.

For the experiment, we placed each larva in an individual flask with approximately 250 mL of stream water. During the experimental period, the larvae were maintained at a temperature of $18 \pm 1^\circ\text{C}$, a photoperiod of 12/12 hours and oxygen saturation $> 90\%$ ($\sim 8 \text{ mg L}^{-1}$). For each larva, we used two leaf disks, one of which remained in a 0.5-mm mesh litter bag (control, without access to the larva) and the other available for consumption. Every 24 h, we checked the containers, and if necessary, the discs were replaced. After 7 days, we removed the larvae and determined their biomass by drying them at $40^\circ\text{C}/48 \text{ h}$. The set of discs offered to the larvae and the control discs were dried ($40^\circ\text{C}/48 \text{ h}$) to determine the consumption rate, based on the following equation (adapted from, Zubro et al. 2011):

Consumption rate ($\text{mg day}^{-1} \text{ mg}^{-1} = [(\text{control disc} - \text{disc consumed})/\text{time}]/\text{biomass}$).

Decomposition rate

We evaluated the decomposition rate based on the results obtained in the stages of microbial conditioning and the consumption of litter by *Phylloicus* sp. To obtain the decomposition rate values, we used the following equation:

$$k = ((P1-P2) - (P1-P2) * P3)/d,$$

where k is the decomposition rate; P1 is the sum of the control discs for each treatment; P2 is the sum of the remaining discs from each treatment; P3 is the correction factor for the leached values from the initial weight of the discs before the experiment; d is the period expressed in days of the experiment.

Data analysis

Initially, we evaluated the assumptions of normality and homoscedasticity; the data sets that did not meet these conditions were log-transformed. To assess the variations in the rates of sporulation and decomposition and the consumption of the discs by the larvae, we used analysis of variance (one-way ANOVA) with Tukey's test a posteriori. Statistical analyses were performed using the version 3.6.3 of the R software (R Core Team, 2020).

Results

Effect of metals on microbial conditioning

In the experiment, we identified 19 species of aquatic hyphomycetes. The species *Lunulospora curvula*, *Flagellospora curvula*, *Aquanectria submersa* and *Anguillospora* sp. were identified in all treatments (Supplementary Material 1).

The sporulation rate differed between the control treatment and the treatments with Cu and Cd (Fig. 1). The sporulation rate of the control treatment was 20.5 ± 7.5 (mean $\pm$ SE) conidia mg^{-1} DM d^{-1} . The sporulation rate ranged from 0.4 to 2.1 mg^{-1} DM d^{-1} conidia in Cu10 and from 11.1 to 221.9 mg^{-1} DM d^{-1} conidia in Cu25. Regarding Cu, the Cu10 treatment differed significantly from the control treatment and the Cu25 treatment, with the latter two treatments being similar ($F_{(2,11)} = 12.4$, $p = 0.001$). The sporulation rate ranged from 1.2 to 84.1 conidia mg^{-1} DM d^{-1} in Cd10 and from 0.8 to 2.7 conidia mg^{-1} DM d^{-1} in Cd25. For Cd, we observed a difference only between the control treatment and Cd25 ($F_{(2,11)} = 5.8$, $p = 0.001$). Finally, the sporulation rate in the mixture treatments was lower in the Cu + Cd 1:1

treatment (1.0 ± 1.8 conidia mg^{-1} DM d^{-1}) and higher in the Cu + Cd 1:2 treatment (3.3 ± 5.9 conidia mg^{-1} DM d^{-1}). The control treatment differed from all mixture treatments ($F_{(3,15)} = 8.8$, $p < 0.001$).

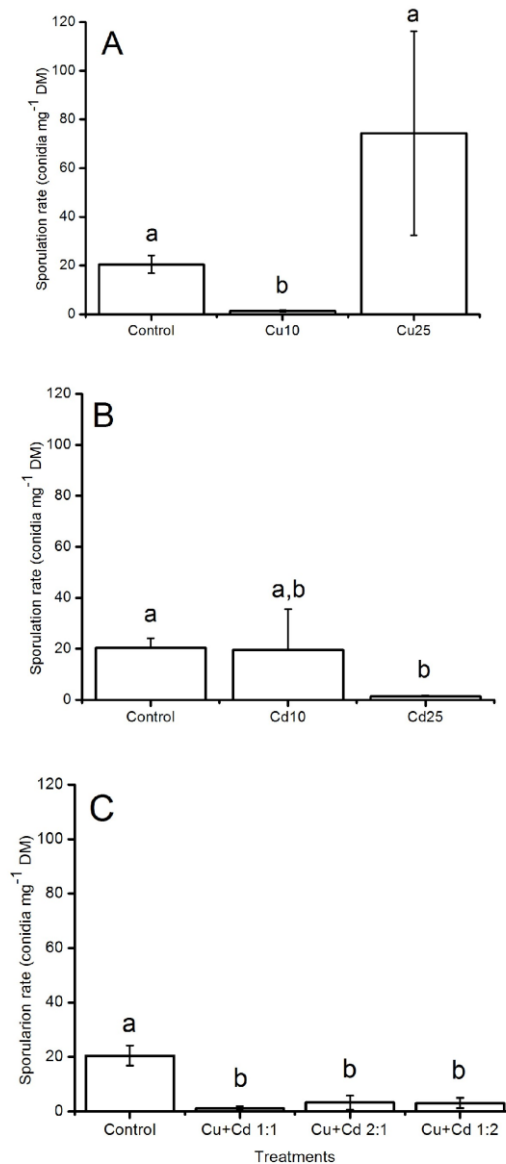


Fig. 1. Rates of sporulation of hyphomycetes in the control, Copper (Cu10 and Cu25) and Cadmium (Cd10 and Cd25) treatments and Mixtures of Cu + Cd (1:1, 2:1 and 1:2). Different letters in the columns represent a significant difference ($p < 0.05$). Comparisons were made between treatments and control separately.

Effect of metals on litter consumption

The larvae survival rate was 78%, with 15% of the larvae soaked and only 7% died. The average consumption of leaf discs in the control treatment was 0.96 ± 0.11 mg day^{-1} mg^{-1} . We observed a reduction in consumption in metal treatments compared to the control. In the treatment with Cu, the average consumption varied from 0.01 to 0.98 mg day^{-1} mg^{-1} in Cu10

and from 0.04 to 0.24 mg day⁻¹ mg⁻¹ in Cu25. For Cu, we observed a difference between the control treatment and the Cu10 and Cu25 treatments ($F_{(2,19)} = 28.4$, $p < 0.001$). For Cd, the average consumption varied from 0.08 to 0.46 mg day⁻¹ mg⁻¹ in Cd10 and from 0.05 to 0.97 mg day⁻¹ mg⁻¹ in Cd25, with differences between the control treatment and the Cd10 and Cd25 treatments ($F_{(2,21)} = 20.4$, $p < 0.001$). For the mixture treatments, the Cu + Cd 2:1 treatment showed the lowest consumption rate (0.11 ± 0.01 mg day⁻¹ mg⁻¹) and the Cu + Cd 1:2 treatment the highest consumption rate (0.21 ± 0.07 mg day⁻¹ mg⁻¹). The control treatment differed from all mixture treatments ($F_{(3,26)} = 43.5$, $p < 0.001$) (Fig. 2).

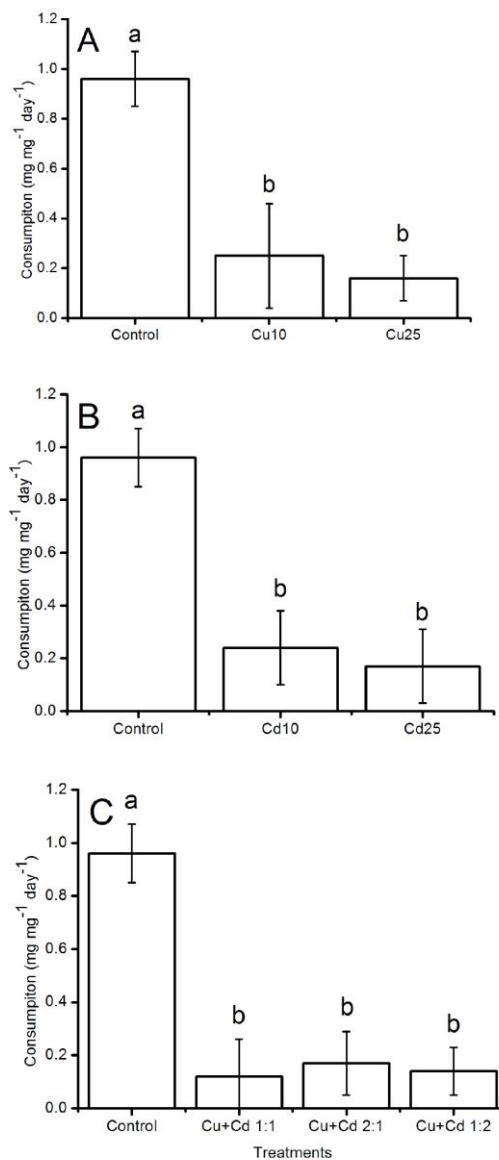


Fig. 2. Consumption of leaf discs by *Phylloicus* in the control, Copper (Cu10 and Cu25) and Cadmium (Cd10 and Cd25) treatments and Mixtures of Cu + Cd (1:1, 2:1 and 1:2). Different letters in the columns represent a significant difference ($p < 0.05$). Comparisons were made between treatments and control separately.

Effects of metals on decomposition rates

The decomposition rates showed a significant reduction compared to the control treatment (Fig. 3). The rate of decomposition in the control treatment was $0.0025 \pm 0.0003 \text{ day}^{-1}$. The treatments Cu10 and Cu25 were similar, but with lower rates compared to the control ($F_{(2,19)} = 19.7$, $p < 0.001$). In the treatment with Cd, the treatments Cd10 and Cd 25 were similar, but with lower rates than in the control ($F_{(2,21)} = 15.8$, $p < 0.001$). For the mixture treatments, the same pattern was observed ($F_{(3,30)} = 18.1$, $p < 0.001$).

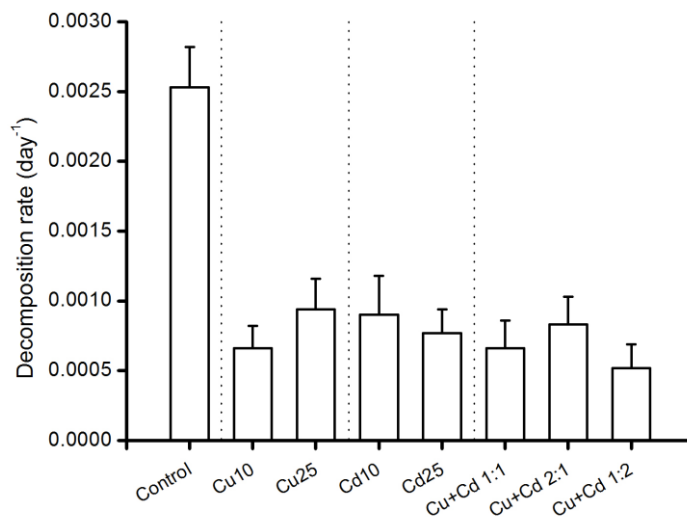


Fig. 3. Decomposition rates (day^{-1}) in the control treatments, Copper (Cu10 and Cu25), Cadmium (Cd10 and Cd25) and Mixtures of Cu + Cd (1:1, 2:1 and 1:2). Different letters in the columns represent a significant difference ($p < 0.05$). Comparisons were made between treatments and control separately.

Discussion

Increasing Cu and Cd concentrations in aquatic environments can affect aquatic communities and, consequently, the ecological processes that they play in the ecosystem. In this study, we investigated the effects of increased concentrations of Cu and Cd on the rates of sporulation of hyphomycetes, consumption by shredders and leaf decomposition in microcosms. Both Cu and Cd influenced all studied stages. Despite the importance of hyphomycetes in increasing leaf palatability for shredders (Biasi et al., 2019), the effects of Cu and Cd associated with sporulation and the consumption rates of *Phylloicus* sp. seem to respond independently to contamination with both metals. Thus, even though the sporulation

rates were not affected (e.g., Cd10 and Cu25), the metals inhibited the feeding activities of the shredders, consequently reducing the rates of leaf decomposition.

Sporulation rate

The magnitude of the effect of metals on the reproductive activity of hyphomycetes depends on the metal type. In this study, the production of conidia was already affected at the lowest concentrations of Cu, but only at the highest concentrations of Cd. The presence of an anthropogenic stressor can affect the microbial activity in streams (Roussel et al., 2008; Azevedo & Cássio 2010); consequently, these effects are translated into possible changes in ecosystem processes (e.g., decomposition). The reproductive activity of hyphomycetes is generally restricted in waters with high metal contamination (Krauss et al., 2001; Solé et al., 2007), leading to a decrease in fungal diversity and altering species dominance (Duarte et al., 2004). However, the ability of hyphomycetes to survive under such conditions, especially in moderately contaminated streams, depends on physiological adaptations, morphological changes and environmental changes in relation to speciation, availability, concentration and toxicity of metals (Gadd, 2007). Increased concentrations of Cu and Cd can induce the formation of free radicals and reactive oxygen species (Valko et al., 2005). However, hyphomycetes have a series of antioxidant molecules and enzymes that, in low concentrations, protect individuals (Braha et al., 2007).

In contrast, at low concentrations, essential and non-essential metals can have a stimulating effect on the reproduction and growth of various aquatic species (Batista et al., 2012; Sridhar & Bärlocher 2011). In our study, the higher concentration of Cu stimulated the sporulation rates of hyphomycetes, contrary to our expectations. Many fungi, including hyphomycetes, can develop tolerance to heavy metals, including reduced adsorption, increased excretion and higher immobilisation of the metal (Ferreira et al., 2016). Intraspecific variations in the hyphomycete community have already been recorded against concentrations of uranium (Bergmann & Graça 2020) and Cd (Fernandes et al., 2011), although further studies are needed to elucidate these issues.

Exposure to Cd at 10× above the natural concentration ($\sim 9.0 \mu\text{g L}^{-1}$) did not affect the sporulation rate of aquatic hyphomycetes. However, at concentrations 25× ($\sim 22.5 \mu\text{g L}^{-1}$) above baseline Cd concentrations, we observed a $\sim 90\%$ decrease in sporulation rates of hyphomycetes. High concentrations of Cd have already been reported to inhibit the reproductive activity of hyphomycetes (Moreirinha et al., 2011), especially as Cd affects the

enzymatic performance of hyphomycetes and, consequently, the use of the resource intended for reproduction (Bergman & Graça 2020). In addition, fungi that resisted the Cu10 and Cd25 treatments may be investing energy in defense mechanisms against metallic stress instead of reproducing (Medeiros et al., 2010). Thus, the reduction in sporulation rates in the Cu10 and Cd25 treatments indicates negative effects over time for aquatic hyphomycete communities. The mixture of Cd + Cu inhibited microbial activity in all combinations; however, in the proportion of Cd + Cu 1:1 of both stressors, the effect was more toxic. The combined effects of metals generally induce complex effects on aquatic communities (Ferreira et al., 2016), which can be additive or antagonistic (Duarte et al., 2008). In our study, the combined effects of Cu + Cd were more pronounced than those of single metals, which corroborates previous studies with a mixture of different metals, such as Cu and Zn (Azevedo et al., 2007; Duarte et al., 2008).

Effect of metals on consumption

Leaf consumption by shredders is a vital process in the flow of energy in streams (Leberfinger & Bohman 2011). In this sense, the rates of detritus consumption by shredders can be used with a functional response proxy in streams (Pradham et al., 2020). In our study, the increase in the concentrations of Cu and Cd negatively affected the leaf consumption by *Phylloicus* sp. in all treatments compared to the control. We observed a reduction of more than 60% in consumption rates in all treatments with the presence of Cu, Cd and the mixture Cu + Cd, corroborating previous studies. For example, Pradhan et al. (2012) found a reduction of 47% in the consumption by shredders of the species *Allogamus ligonifer* in water contaminated with Cu nanoparticles. For Cd, Batista et al. (2012) found that at concentrations of 35 mg L⁻¹, the consumption rates of shredders of the species *Limnephilus* sp. decreased by up to 60% per leaf. In addition, the concentration studied by Batista et al. (2012) was more than 100 times greater than the highest concentration tested in our study, demonstrating the high impact of this metal on ecosystem patterns and processes.

Shredders can select their resources according to the quality of the detritus, having preferences for soft and conditioned leaves (Graça, 2001). We believe that *Phylloicus* sp. can detect the presence of metals in the detritus and reduces consumption, thereby directly responding to the contaminant (Gonçalves et al., 2011). Although the presence of Cu and Cd did not affect the mortality rates of *Phylloicus* sp. reductions in consumption rates can affect the physiological performance, growth and reproduction of organisms (Batista et al. 2012). In

sublethal concentrations, the increase in metal concentrations can affect the generation of oxidative stress (Barata et al., 2005). Tagliaferro et al. (2018) found that the increase in copper concentrations was related to the increase in catalase in shredder insects. Thus, the effects can be maximised over time leading, to the elimination of sensitive species.

In this study, the increase in the concentrations of Cu and Cd in the environment considerably reduced the consumption rates of shredder insects. As the detritus is considered an important feeding route in small streams, the reductions in the consumption rates of shredders considerably affect the secondary production rates. Woodcock & Huryn (2007) found that in a gradient of pollution by metals, there was a decrease in secondary production in streams, where shredders were one of the most affected functional trophic groups. Still, the decomposition process does not stop in the fragmentation process, and the fine particles are used by other consumers (e.g., collector-collector) (Graça, 2001), thus affecting the energy flow in these environments.

Effects of metals on decomposition rates

The inhibition of litter consumption by shredders, caused by exposure to metals, affected the decomposition rates. Our results indicate that the decomposition rates were at least 3× higher in the control treatment. Considering that leaf consumption is the main source of energy for trophic chains in forested streams (Vannote et al., 1980), and secondary production is dependent on this resource (Graça, 2001), metals in aquatic environments can affect the functioning of these environments.

As previously mentioned, in our study, metals negatively affected sporulation rates, consumption by shredders and decomposition rates. The effects of metal mixing on the leaf decomposition process in streams are still poorly understood. Although the relative concentrations of metal mixtures can change under natural conditions (Norwood et al., 2003), laboratory experiments can help to understand the effects generated by mixing two or more metals on the functioning of aquatic environments. Although Cu and Cd perform different metabolic functions in organisms, our results demonstrate that the simultaneous increase in the concentrations of Cu and Cd accentuates the biological stages of decomposition compared to the individual effects. Although there are no studies in the literature on the effects of Cu and Cd in combination on shredder insects, the effects of different mixtures can vary. For example, Fernandes et al. (2009) found that the combination of zinc + phosphate affected the community composition of fungi in decomposing leaves. Similarly, Moreirinha et al. (2011)

observed that the effects of Cd on fungal biomass and sporulation rates were accentuated with the presence of phenanthrene. However, Abel & Bärlocher (1984) found that Cd toxicity in an aquatic hyphomycete community decreased with the presence of calcium and magnesium ions.

Conclusions

In forested streams, the leaf decomposition process is directly linked to the functioning of these environments. The increase in Cu and Cd concentrations negatively affected the biological stages of the decomposition process, despite microbial conditioning, which facilitates the fragmentation process. The presence of metals independently influenced the different stages of decomposition. Our results indicate that the negative effects observed on litter consumption by shredders are directly reflected in the leaf decomposition rates. At the highest concentrations of Cu and at intermediate Cd concentrations, the sporulation rates of aquatic hyphomycetes were higher in relation to the control, which may be an important strategy for the dispersion of some species of hyphomycetes in the context of an anthropogenic stressor. For shredders, we found that the presence of Cu and/or Cd significantly reduced consumption rates and, consequently, influenced leaf decomposition rates. Shredders are responsible for transforming organic matter into small particles, which serve as a food source for other functional trophic groups (e.g., collectors). Thus, in addition to reducing decomposition rates, this can affect the distribution of resources to other trophic groups.

The sporulation rate is considered a measure of microbial activity and is currently related to decomposition rates. Thus, we suggest that in streams contaminated by metals, there is a decrease in carbon being directed to fungal reproduction. Consequences, such as species loss, can be translated into changes in decomposition rates if the species that remain are not able to functionally compensate sensitive species. In addition, our results demonstrate that the increase in Cu + Cd concentrations increases the negative effects on the decomposition processes. Although this was a laboratory experiment, it is known that aquatic environments are vulnerable to human activities, and the increase in metal concentrations is already a concern. Thus, our results help to understand the effects of metals in the stages of decomposition and, further, demonstrate that such studies can be used to understand the effects on aquatic biota.

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Supplementary material 1. Aquatic hyphomycete species identified in control treatments, Cu10, Cu25, Cd10, Cd25, Cu+Cd 1:1, Cu+Cd 2:1 e Cu+Cd 1:2.

Espécies	Control	Cu10	Cu25	Cd10	Cd25	Cu+Cd 1:1	Cu+Cd 2:1	Cu+Cd 1:2
<i>Alatospora acuminata</i> Ingold, 1942	X	X		X				X
<i>Alatospora pulchella</i> Marvanová, 1977	X	X	X			X		
<i>Anguillospora</i> sp.	X	X	X	X	X	X	X	X
<i>Anguillospora filiformis</i> Greath., 1961	X		X	X		X		
<i>Aquanectria submersa</i> (H.J. Huds.) L. Lombard & Crous, 2015	X	X	X	X	X	X	X	X
<i>Articulospora tetracladia</i> Ingold, 1942	X	X		X	X	X		X
<i>Campylospora</i> <i>chaetocladia</i> Ranzoni, 1953	X	X	X	X				X
<i>Campylospora filicladia</i> Nawawi, 1974								X
<i>Clavariopsis aquatica</i> De Wild, 1895				X				X
<i>Flagellospora curvula</i> Ingold, 1942	X	X	X	X	X	X	X	X
<i>Heliscus tentaculus</i> Umphlett, 1959	X	X	X	X			X	X
<i>Lemonniera labamensis</i> R.C. Sinclair & Morgan- Jones 1979								
<i>Lemonniera</i> <i>pseudofloscula</i> Dyko 1977			X					X
<i>Lunnulospora curvula</i> Ingold, 1942	X	X	X	X	X	X	X	X
<i>Mycocentrospora clavata</i> S.H. Iqbal 1974						X		
<i>Tetracladium</i> <i>marchalianum</i> De Wild, 1893		X		X			X	
<i>Tripospermum myrti</i> (Lind) S. Hughes, 1951		X						
<i>Triscelophorus</i> <i>acuminatus</i> Nawawi, 1975	X	X						
<i>Triscelophorus</i> <i>monosporus</i> Ingold, 1943		X		X	X			X

CAPÍTULO 4

Mudanças comportamentais em insetos predadores na presença de metais



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Mudanças comportamentais em insetos predadores na presença de metais

Resumo

O aumento das concentrações de metais nos ambientes aquáticos vem sendo reportado em várias regiões do mundo. Muitas espécies consideradas tolerantes podem ter seu comportamento afetado diante desses poluentes. A partir disso, o objetivo desse trabalho foi avaliar as atividades comportamentais de larvas de *Lestes* sp. (predador) durante a predação de larvas de *Chironomus* sp. (presa) em diferentes cenários de contaminação de cobre (Cu) e cádmio (Cd). Para isso, desenvolvemos o estudo sob condições controladas, onde avaliamos os comportamentos tempo de captura da presa, procura, tentativas de captura e mobilidade por larvas de *Lestes* sp. Para determinar as concentrações dos metais, utilizamos as concentrações basais de um riacho referência e tratamentos onde aumentamos as concentrações de ambos os metais em 10× e 25×. Nossos resultados indicam que o aumento das concentrações de Cu e Cd no ambiente afetaram o comportamento de predação de *Lestes* sp. Verificamos que a mobilidade e a procura de *Lestes* sp. foram reduzidos nos tratamentos com Cu e Cd. O tempo de captura e as tentativas de capturas das presas foram significativamente maiores nos tratamentos com os metais em comparação com o tratamento controle. Assim, os efeitos observados neste estudo podem refletir no desenvolvimento, emergência e maturação dos predadores e, desta forma, influenciar negativamente nas cadeias tróficas aquáticas.

Palavras-chave: Odonata; predador-presa; cobre; cádmio

Introdução

As Odonata (Insecta) são insetos hemimetábolos importantes na cadeia trófica dos ecossistemas aquáticos (Kalkman et al. 2008). As larvas de Odonata são classificadas com predadores intermediários se alimentando principalmente invertebrados, além de pequenos vertebrados, como pequenos anfíbios e peixes (Stoks and Crdoba-Aguilar 2012). Em contra partida, as larvas de Odonata são um importante recurso alimentar para aves, artrópodes, peixes e anfíbios (Souza et al. 2007). Além disso, as Odonata são cosmopolitas, habitando praticamente todos os tipos de ambientes de água doce (Corbet 1999). Como um importante representante na fauna de insetos aquáticos, as larvas de Odonata vem sendo utilizadas em diversos programas de monitoramento ambiental, servindo de modelo para avaliar a biodiversidade, qualidade da água e funcionamento do ecossistema (Stoks and Crdoba-Aguilar 2012; Nasirian and Irvine 2017).

Neste sentido, o aumento das concentrações por metais tem se tornado uma preocupação crítica pela sua toxicidade e seu potencial de acumulação no ambiente (Kumar et al. 2019; Loureiro and Hepp 2020). A utilização de biomonitores vem sendo considerada uma ferramenta eficaz para avaliar a contaminação em ambientes aquáticos (Nasirian and Irvine 2017; Loureiro et al. 2018). A ampla distribuição e a capacidade em acumular e tolerar elevadas concentrações de metais, faz com que as larvas de Odonata sejam consideradas um excelente modelo biológico na avaliação de ambientes contaminados por metais (Tollett et al. 2009; Fletcher et al. 2020). Tollett et al. (2009) verificaram que as larvas de Odonata foram capazes de acumular $>1000 \mu\text{g g}^{-1}$ de peso seco de cádmio, chumbo e cobre em ambientes sob altas concentrações destes metais. Corbi et al. (2008) verificaram uma relação positiva entre o aumento das concentrações de metais no sedimento e nas larvas de Odonata quando associadas a ambientes circundados por atividades agrícolas. Além de acumular metais, as larvas de Odonata podem ser responsáveis pela transferência desses elementos para o ambiente terrestre (Corbi et al. 2008), uma vez que essa ordem contribui com grande quantidade de biomassa após a emergência dos adultos (Popova et al. 2016).

Além das características das comunidades biológicas, o comportamento animal vem sendo utilizado como um indicador de mudanças ambientais (Mogren and Trumble 2010). O comportamento animal é o conjunto de respostas em nível de indivíduo sobre um determinado tipo de estresse (e.g. contaminantes ambientais). Estas respostas comportamentais apresentam relações claras com processos bioquímicos (e.g. efeitos neurológicos) e seus resultados podem ser usados em nível de população e comunidades (Weis et al. 2001). Para os insetos aquáticos,

atividades comportamentais como predação, locomoção e reprodução podem gerar informações importantes para as interações ecológicas dos ambientes aquáticos (Mogren and Trumble 2010). Brooks et al. (2009) verificaram que a contaminação por cádmio não alterou a quantidade de presas consumidas por larvas de *Ischnura elegans* (Odonata), porém, foi observada a preferência por larvas vivas em comparação as mortas. Finotello et al. (2017) verificaram que larvas de *Ischnura graellsii* (Odonata), quando expostas ao inseticida Lambda Cialotrina, diminuía significativamente o sucesso de captura de suas presas. Desta forma, fica evidente a importância de conhecer as reações comportamentais de organismos aquáticos em ambientes sujeitos à contaminação por elementos químicos.

Metais como cobre (Cu) e cádmio (Cd) vêm ganhando ampla atenção na comunidade científica e de gestores públicos, pelo potencial tóxico às comunidades aquáticas e a seres humanos (Mebane 2010; Hadjipanagiotou et al. 2020; Loureiro and Hepp 2020). Como as Odonata conseguem suportar condições elevadas de concentrações de Cu e Cd (Tollett et al. 2009; Nasirian and Irvine 2017), os danos destes metais sobre estes organismos podem ocorrer sobre características comportamentais (e.g. mudanças na locomoção, alimentação e reprodução) (Mogren and Trumble 2010). Sendo assim, neste estudo, avaliamos as atividades comportamentais de larvas de *Lestes* sp. (Insecta, Odonata) durante a predação de larvas de *Chironomus* sp. (Insecta, Diptera) em diferentes cenários de contaminação de Cu e Cd. Trabalhamos com concentrações subletais de ambos metais, permitindo assim, que encontrássemos respostas mais sutis à contaminação ambiental quando comparada com abordagens toxicológicas tradicionais (Boyd 2010). Nossas hipóteses foram que os tratamentos com Cd e as maiores concentrações de Cu afetariam negativamente os comportamentos de locomoção, tempo de captura, procura e tentativas de captura da presa. Essa expectativa ocorre devido à característica química de que o Cu é um metal essencial enquanto que o Cd é um metal não essencial (Mogren and Trumble 2010). Desta forma, apenas as concentrações maiores de Cu irão causar efeitos marcantes sobre o comportamento das larvas de Odonata.

Material e métodos

Este estudo foi conduzido sob condições controladas em microcosmos considerando quatro etapas sequenciais: (i) coleta do material biótico e abiótico em um riacho, (ii) aclimação dos organismos em laboratório, (iii) preparação dos tratamentos e (iv) testes comportamentais.

Utilizamos larvas do gênero *Lestes* sp. (Odonata, Zygoptera, Lestidae) como predador modelo. As larvas de *Lestes* sp. (chamadas a partir de agora por *Lestes*) foram coletadas em um lago artificial localizado em Erechim, no norte do Rio Grande do Sul (27°61'14"S e 52°23'01"O). Como presa, utilizamos larvas do gênero *Chironomus* sp. (Diptera, Chironomidae; chamadas a partir de agora por *Chironomus*) coletadas em um riacho de 2º ordem localizado no mesmo município (27°62'11"S e 52°24'26"O). Coletamos as larvas de *Lestes* e *Chironomus* com o auxílio de um amostrador Surber (250 µm malha; 0,09 m² área) e, em seguida, transportamos os organismos em caixas com um pouco de água dos ambientes ao laboratório. Para a realização do experimento, utilizamos água de um riacho de 2º ordem (27°48'35"S and 52°30'09"O) com ausência aparente de perturbações antrópicas e com vegetação ripária característica de Mata Atlântica em ambas as margens.

No laboratório, as larvas de *Lestes* sp. e as larvas de *Chironomus* sp. foram mantidas separadamente em aquários com água do riacho por 24 horas para aclimação. Os aquários permaneceram em temperatura ambiente controlada ($18 \pm 1^\circ\text{C}$), fotoperíodo de 12/12 horas (luz/escuro) e com saturação de oxigênio $>90\%$ ($\sim 8 \text{ mg L}^{-1}$).

Organizamos cinco tratamentos com diferentes concentrações de Cu e Cd. Determinamos as concentrações basais de Cu e Cd a partir das concentrações médias observadas no riacho ($\text{Cu} \sim 1,6 \text{ } \mu\text{g L}^{-1}$ e $\text{Cd} \sim 0,9 \text{ } \mu\text{g L}^{-1}$; Loureiro et al., 2021). A partir destas concentrações definimos o *tratamento controle* apenas com água do riacho, tratamentos 'Cu10' e 'Cd10' com aumento de $10\times$ a concentração inicial dos respectivos metais. Para os tratamentos 'Cu25' e 'Cd25' aumentamos $25\times$ a concentração inicial de ambos os metais. A adição dos metais para preparo dos tratamentos foi realizada a partir da diluição de soluções de Cu e Cd, utilizamos amostras padronizadas de 1000 mg L^{-1} (Merck 1.09987.0001 e Merck 1.09960.0001, respectivamente). Para cada tratamento preparamos sete réplicas, contendo larvas de *Lestes* individualmente em copos plásticos de 100 mL contendo 70 mL da solução do respectivo tratamento por 24 horas em temperatura controlada ($18 \pm 1^\circ\text{C}$) e fotoperíodo de 12/12 horas (luz/escuro).

O comportamento de cada larva de *Lestes* foi observado duas vezes num intervalo de 24 horas, totalizando 70 observações (unidades amostrais). Para avaliação do comportamento das larvas de *Lestes*, no momento da observação, colocamos uma larva de *Chironomus* em cada copo e acompanhamos as ações por 300 segundos. As atividades comportamentais avaliadas foram: (i) tempo de captura da presa (tempo gasto para a captura da presa), (ii) procura (movimentação em direção a presa), (iii) tentativas de captura (tentativas de capturas pelo predador até obter sucesso de captura) e (iv) mobilidade (quantidade de movimentos feitos

pelo predador sem intenção de pegar a presa). Classificamos a mobilidade das larvas em 3 categorias: (i) parada (a larva não se movimentou a não ser para capturar a presa), (ii) lento (a larva realizou poucos movimentos no máximo 3 movimentos) e (iii) rápido (a larva realizou ao menos 4 ou mais movimentos). Após o experimento, confirmamos a identificação taxonômica das larvas de *Lestes* sp. a partir da chave de identificação taxonômica de Souza et al. (2007) e as larvas de *Chironomus* sp. a partir da chave de identificação taxonômica de Trivinho-Strixino (2011).

Verificamos a variação nos tempos de captura entre as larvas de *Lestes* a partir de uma análise de Kruskal-Wallis com um teste Dunn *a posteriori*. As atividades comportamentais de procura, captura e mobilidade foram avaliadas a partir da comparação entre as proporções observadas e esperadas em cada tratamento com o uso de um teste de Qui-Quadrado. Em todas as análises comparamos os tratamentos com metais em relação ao tratamento controle. Realizamos as análises estatísticas utilizando o software BioEstat (versão 5.3).

Resultados

A taxa de sobrevivência das larvas de *Lestes* foi de 74,5% ao final do experimento. O tempo médio de captura das larvas de *Lestes* foi de $82,1 \pm 18,8$ segundos no tratamento controle. O tempo de captura das larvas nos tratamentos Cu10 ($80,3 \pm 25,4$ s) e Cu25 ($111,6 \pm 39,7$ s) foram semelhantes ao tempo de captura no tratamento controle (Fig. 1A). Por outro lado, as larvas de *Lestes* nos tratamentos com Cd apresentaram maior tempo de captura das presas. O tempo médio na captura das presas foi de $246,5 \pm 36,2$ s no tratamento Cd10 e de $183,5 \pm 36,7$ s no tratamento Cd25 (Fig. 1B). Porém, observamos diferença no tempo de captura apenas entre o tratamento controle e o tratamento Cd10 ($H=9,7$, $df=2$, $p=0,007$) (Fig. 1B).

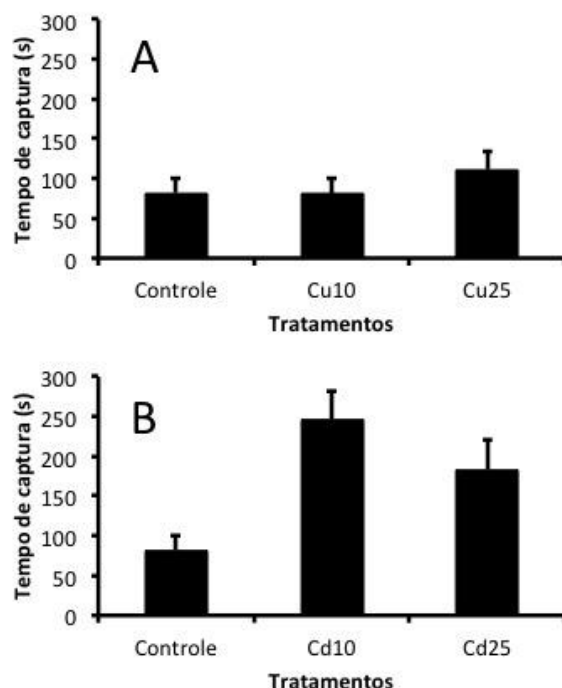


Fig 1. Tempo de captura das larvas de *Lestes* sp. sobre *Chironomus* sp. nos diferentes tratamentos com Cobre (Cu10 e Cu25) e Cadmio (Cd10 e Cd25).

Os comportamentos de procura, tentativa de captura e mobilidade das larvas de *Lestes* variaram entre os tratamentos. A procura das larvas de *Lestes* reduziu significativamente nos tratamentos Cu25 ($\chi^2=6,9$, $p=0,008$) e Cd10 ($\chi^2=25$, $p<0,001$) em comparação ao tratamento controle (Fig. 2A). No tratamento controle, 80% das larvas de *Lestes* realizaram o comportamento procura em direção à presa. Por outro lado, nos tratamentos Cu10 e Cu25, a proporção do comportamento procura foi de 67% e 50% respectivamente, enquanto que nos tratamentos Cd10 e Cd25 a atividade de procura pela presa foi de 29% e 60%, respectivamente.

O sucesso de captura das larvas de *Chironomus* por *Lestes* foi de 100% no tratamento controle. Assim, observamos uma redução significativa de sucesso de captura das larvas com os demais tratamentos (Fig. 2B). Para os tratamentos Cu10 e Cu25, as proporções na atividade captura foram de 67% para ambos os tratamentos ($\chi^2=6,5$, $p=0,001$ e $\chi^2=6,9$, $p<0,010$ respectivamente). Nos tratamentos Cd10 e Cd25 observamos uma proporção na captura de 43% ($\chi^2=22,7$, $p<0,001$) e 60% ($\chi^2=10$, $p=0,001$), respectivamente.

A mobilidade das larvas de *Lestes* reduziu significativamente nos tratamentos com Cu e Cd em comparação com o tratamento controle (Fig. 2C). Observamos 90% das larvas com mobilidade nas categorias parado e lento no tratamento Cu10, enquanto que observamos 100% das larvas nestas duas categorias no tratamento Cu25. Por sua vez, em ambos os tratamentos com Cd, a proporção de larvas nas categorias parado e lento foi de 90% (Fig. 2C).

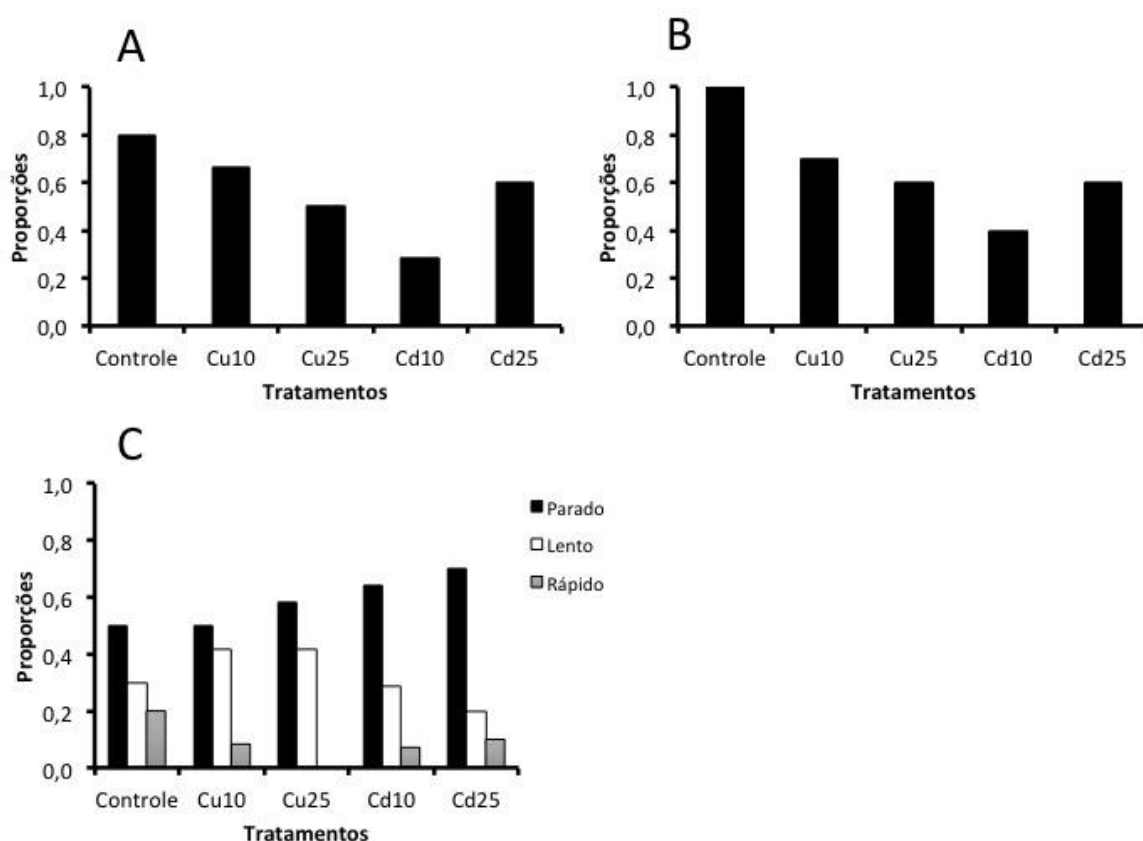


Fig. 2 Proporções dos comportamentos de (A) procura, (B) tentativa de captura e (C) mobilidade das larvas de *Lestes* entre os diferentes tratamentos com cobre (Cu10 e Cu25) e cádmio (Cd10 e Cd25).

Discussão

Neste estudo, verificamos que microcosmos com concentrações mais elevadas de Cu e Cd alteram o comportamento de predadores do gênero *Lestes* durante sua relação com presas vivas. Apesar de o experimento ter sido realizado sob concentrações controladas e as concentrações testadas, tanto para Cu e Cd, são semelhantes às concentrações destes mesmos metais observadas em ambientes aquáticos da região (Loureiro et al. 2018; Borges et al.

2021). Sendo assim, os efeitos avaliados neste estudo podem ser extrapolados para ambientes naturais, uma vez que as concentrações testadas aqui podem ocorrer principalmente em rios ou riachos associados a atividades antrópicas (Loureiro et al. 2018, 2021).

Nossos resultados demonstraram efeitos distintos sobre o comportamento de *Lestes*, contrariando, em parte, nossas expectativas. Em ambientes aquáticos, alterações nas concentrações normais dos metais no ambiente podem estimular ou simplesmente não exercer efeitos comportamentais nos organismos (Gosselin and Hare 2004; Dornfeld et al. 2009). Ainda, um efeito positivo ou negativo associado ao comportamento animal não necessariamente confere uma vantagem ou desvantagem ao indivíduo (Mogren and Trumble 2010). Por exemplo, em testes laboratoriais, Hare and Shooner (1995) verificaram que larvas de *Chironomus* não evitavam sedimentos contaminados por Cd. Além disso, os autores observaram que em locais contaminados por Cd, a baixa densidade de larvas poderia estar associada a toxicidade do elemento e não a um efeito sobre o comportamento dos animais. Gerhardt et al. (2005) verificaram que em locais com elevadas concentrações de Cu houve um aumento significativo na locomoção de larvas de *Choroterpes picteti* (Ephemeroptera, Leptophlebiidae), atribuindo isso a tentativa de fuga dos organismos para ambientes menos contaminados. Deste modo, embora parte de nossas expectativas tenham sido refutadas, a literatura demonstra que diferentes metais (essenciais ou não essenciais) podem gerar efeitos distintos sobre os diferentes organismos aquáticos.

A exposição ao Cd aumentou em $\sim 3\times$ o tempo de captura das lavas em relação ao tratamento controle. A interação predador-presa é uma relação que consiste em diversas estratégias para a sobrevivência, onde a detecção precoce é fundamental para o sucesso de ambas partes (Ferrari et al. 2010). Assim, a demora em capturar a presa pode gerar uma demanda maior de energia por indivíduo capturado, refletindo em efeitos negativos ao longo do ciclo de vida do predador. Stoks and Aguilar (2012) destacam que restrições na alimentação, altas temperaturas e risco de predação afetam diretamente a fase de vida adulta de Odonata. Desta forma, os efeitos dos metais sobre os organismos aquáticos podem ocorrer ao longo do ciclo de desenvolvimento das larvas e não de forma imediata.

A capacidade do comportamento procura das larvas de *Lestes* em procurar as presas, diminuiu nas maiores concentrações de Cu e nas menores de Cd. O comportamento procura pode ser determinado pelos benefícios entre o ganho de energia e o risco de mortalidade por predação ou outras causas (Hirvonen and Ranta 1996). A redução em procurar a presa pode influenciar no sucesso de captura de presas e também na chance de serem predados por outros organismos. Assim, a redução da capacidade de procurar a presa pode ter um efeito negativo

(diminuição no encontro de presas) e positivo (redução da exposição para predadores). Esses argumentos são corroborados quando observamos os resultados relacionados ao sucesso de captura das larvas de *Lestes*. As larvas de *Lestes* tiveram um aproveitamento de 100% no tratamento controle e uma redução significativa nos demais tratamentos. Para as Odonata, as pistas visuais e a presença de mecanorreceptores são fundamentais para a captura das presas (Rebora et al. 2004). Ainda, a utilização de movimentos rápidos para capturar as presas apresenta desafios em termos de movimentos devido às forças de arrasto (Crumrine 2006). Assim, o aumento das concentrações de Cu e Cd nas tentativas de capturas de presas por larvas de *Lestes* podem ter afetado as relações predador-presa de maneira: (i) direta, onde o gasto energético para a captura de um recurso é maior e (ii) indireta, onde as tentativas mal sucedidas podem representar um risco as presas, uma vez que as que os ataques podem ocasionar lesões aos indivíduos, gerando redução nas populações de presas. Assim, o aumento das tentativas nas capturas de presas por larvas de *Lestes* pode levar a um efeito subletal do predador, uma vez que os palpos labiais tem potencial de lesionar as presas (e.g. anfíbios) (Bowerman et al. 2010).

O aumento das concentrações de Cu e Cd reduziu a locomoção das larvas de *Lestes*. Em ambientes lóticos, algumas espécies aquáticas podem aumentar a sua locomoção como uma estratégia de evitar os locais contaminados (Gerhardt 1998; Mogren and Trumble 2010). A dinâmica do nosso experimento não permitiu esse tipo de avaliação pois foi realizado em um sistema de fluxo fechado. No entanto, a presença de larvas de Odonata em locais contaminados por Cu e Cd é bem reportada na literatura (Tollett et al. 2009; Nasirian and Irvine 2017). A permanência dos organismos em um ambiente contaminado pode resultar em alterações na locomoção e outros comportamentos (Gerhardt 1998). Essa redução no comportamento de locomoção pode ser uma consequência da alocação na demanda energética para a aclimação dos contaminantes ou desintoxicação (Riddell et al. 2005).

Conclusão

O aumento das concentrações de Cu e Cd afetou o comportamento das larvas de *Lestes* na sua relação predador-presa. Ambos os metais alteraram o comportamento de captura, procura e mobilidade dos animais. De modo geral, as maiores concentrações de Cu (metal essencial) apresentaram algum efeito sobre o comportamento das larvas, enquanto que ambas as concentrações de Cd (metal não essencial) afetaram negativamente o comportamento das larvas. Ainda, a mobilidade das larvas foi mais afetada, o que reflete diretamente sobre os

outros comportamentos avaliados. Nosso estudo demonstra que a presença de metais em ambientes aquáticos causa alterações comportamentais nos animais, reduzindo atividades vitais associadas a alimentação. Desta forma, podemos acreditar que os efeitos dos metais podem ir além da dificuldade de os predadores obterem seu alimento, mas isso pode refletir em aspectos futuros, associados ao desenvolvimento, emergência e maturação.

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CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Os resultados da minha tese mostraram que a abordagem com diferentes grupos tróficos funcionais dos insetos aquáticos foram uma ferramenta útil na avaliação da contaminação de metais em ecossistemas aquáticos. Realizei neste trabalho, diferentes abordagens metodológicas buscando avaliar como as mudanças nas concentrações de cobre e cádmio podem afetar as interações entre os grupos tróficos funcionais e os ambientes aquáticos. Em referência aos objetivos propostos nesta tese, observei que há uma forte relação entre as concentrações dos metais estudados com as interações ecológicas (meio biótico e abiótico) dos diferentes grupos tróficos de insetos aquáticos. Ainda, observei que os metais são facilmente incorporados pelos insetos nas diferentes cadeias tróficas, principalmente pelos seus recursos alimentares, e o metal, uma vez incorporado pelo animal, causa efeitos adversos aos organismos, inclusive comportamentais. Além desses efeitos à nível de organismo, os efeitos dos metais causam alterações no funcionamento dos ambientes.

No primeiro capítulo avaliei como mudanças no ambiente em diferentes escalas (áreas de drenagem e riachos) afetam a incorporação de cobre (Cu) e cádmio (Cd) por duas espécies de insetos raspadores. Verifiquei que as combinações de taxonomia com características funcionais dos organismos podem ser importantes na determinação das relações entre as concentrações de metais no ambiente e a fauna aquática. Conclui que a intensidade das atividades agrícolas na área de drenagem dos riachos influencia as concentrações de metais no ambiente, facilitando a absorção pelo biofilme. Como o biofilme é o principal recurso alimentar dos raspadores, a incorporação dos metais pelos insetos ficou facilitada e, dependendo das características do metal, pode ser biomagnificado para níveis tróficos mais elevados.

No segundo capítulo, avaliei como as mudanças nas concentrações de Cu e Cd podem afetar o comportamento alimentar em insetos fragmentadores e consequentemente, sobre o processo de decomposição de matéria orgânica em riachos. Realizei um experimento em laboratório simulando separadamente as etapas do condicionamento microbiano e a fragmentação biológica por insetos fragmentadores. Conclui que mesmo em ambientes com a presença de detritos (recurso para os fragmentadores) o aumento das concentrações de Cu e Cd afetam as taxas de esporulação dos hifomicetos aquáticos e as taxas de alimentação dos insetos fragmentadores. Consequentemente, as taxas de decomposição foram reduzidas, portanto, além dos efeitos negativos associados aos insetos fragmentadores o aumento nas

concentrações de Cu e Cd no ambiente afeta o processo de decomposição e a distribuição de recursos para outros grupos tróficos funcionais.

No terceiro capítulo, avaliei os efeitos do cobre e cádmio no comportamento de predação por larvas de Odonata em condições controladas. Concluímos que comportamento mobilidade e a procura das larvas de *Lestes* sp. foram reduzidos nos tratamentos com Cu e Cd. O tempo de captura e as tentativas de capturas foram significativamente maiores nos tratamentos com Cu e Cd, podendo significar em uma demanda maior de energia por indivíduo capturado. Assim, destacamos que os efeitos observados neste estudo, podem ir além da dificuldade de os predadores obterem seu alimento, mas isso pode refletir em aspectos futuros, associados ao desenvolvimento, emergência e maturação.

No quarto e último capítulo, realizamos uma revisão bibliográfica sobre a presença dos elementos traço em riachos e os problemas que o aumento destas concentrações pode causar para estes ambientes. Nesta revisão, reunimos informações sobre as principais características dos elementos traço, seus efeitos sobre os organismos aquáticos e as formas de remediação desses impactos. Ainda, verificamos que embora a maioria dos estudos envolvendo este tipo de contaminação esteja associada a fontes pontuais, a expansão agrícola é uma atividade com um alto potencial de contaminação de elementos traço para os corpos hídricos.

Diante dos resultados obtidos com esse trabalho, minha principal conclusão/contribuição é que a utilização dos grupos tróficos funcionais de insetos aquáticos auxilia na compreensão dos efeitos que a contaminação por metais exerce sobre os ambientes aquáticos. De modo geral, a utilização de insetos aquáticos como indicadores de contaminação por metais já é bem evidenciada na literatura. No entanto, nossos resultados fornecem informações importantes sobre as interações entre os insetos aquáticos com os ecossistemas aquáticos, na qual estão associados com o funcionamento destes ambientes. Assim concluo que a presença de metais no ambiente aquático, pode afetar negativamente as rotas alimentares de insetos aquáticos, podendo refletir na diminuição da sua biodiversidade e prestação de serviços ambientais. Ainda, considerando que os insetos aquáticos são organismos que compreendem um nível intermediário da cadeia trófica aquática, a contaminação destes organismos pode afetar os níveis tróficos superiores. Desta forma, os resultados obtidos nesta tese auxiliam na formação de uma base conceitual sólida sobre os efeitos que os metais podem causar ao ambiente aquático, subsidiando assim, a elaboração de estratégias de mitigação dos efeitos antrópicos sobre a biota aquática e processos ecológicos.

No entanto, ainda existem lacunas no que se refere ao uso da abordagem dos grupos tróficos funcionais de insetos em estudos sobre a contaminação de metais em riachos. É

importante compreender as mudanças nas concentrações de metais ao longo do tempo no ambiente e nos diferentes grupos tróficos funcionais de insetos aquáticos. Além disso, uma questão importante não abordada neste trabalho está relacionada com a transferência de metais para o ambiente terrestre. Uma vez que boa parte dos insetos aquáticos possui a sua fase reprodutiva no ambiente terrestre e constitui uma importante fonte de alimento para diversos organismos terrestre. Assim, os insetos aquáticos podem representar uma importante fonte de contaminação de metais na interface ambientes aquáticos e terrestres.

ANEXOS

Anexo 1: Artigos publicado como coautor durante o doutorado em 2017-2021.

TONIN, A. M. et al. Plant litterdynamics in the forest-stream interface: precipitation is a major control across tropical biomes. **Scientific Reports**. v. 7, p. 1–14. 2017. DOI: 10.1038/s41598-017-10576-8

DELIBERALLI, W.; CANSIAN, R. L.; PEREIRA, A. A. M.; LOUREIRO, R. C.; HEPP, L. U.; RESTELLO, R. M. The effects of heavy metals on the incidence of morphological deformities in Chironomidae (Diptera). **Zoologia** v. 35, p. 1–7. 2018. DOI: 10.3897/zoologia.35.e12947

LOUREIRO, R. C.; MENEGAT, M. N.; RESTELLO, R. M.; HEPP, L. U. Incorporation of zinc and copper by insects of different functional feeding groups in agricultural streams. **Environmental Science and Pollution Research**. v. 25, p. 17402- 17408, 2018. DOI: 10.1007/s11356-018-1971-9.

ONGARATTO, R.; LOUREIRO, R. C.; RESTELLO, R. M.; HEPP, L. U. Effects of land use and limnology variables on the dissimilarity of common and rare aquatic insects in Atlantic Forest streams. *Revista de Biología Tropical*. 66: 1223–1231. 2018. DOI: 10.15517/rbt.v66i3.30825

MENEGAT, M. N; LOUREIRO, R. C.; MAGRO, J.; RESTELLO, R. M.; HEPP, L. U. Biodisponibilidade de metais em riachos e a acumulação por insetos aquáticos. **Revista Perspectiva**, v. 44, n. 165, p. 21–33, 2020. DOI: 10.31512/persp.v.44.n.165.2020.71.p.21-33

LOUREIRO, R. C.; MARTINS, M. C.; PEREIRA, A. M. **Contaminação de riachos por metais e seus efeitos sobre macroinvertebrados bentônicos na região Alto Uruguai Gaúcho**. Ecologia de Riachos no Alto Uruguai Gaúcho, Erechim, RS: EdiFAPES, 2020.

