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INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, CONSERVAÇÃO E MANEJO DA VIDA SILVESTRE

A ECOTOXICOLOGIA COMO FERRAMENTA PARA O MONITORAMENTO E PERÍCIA AMBIENTAL EM ÁREAS DE MINERAÇÃO

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Belo Horizonte, MG
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A ECOTOXICOLOGIA COMO FERRAMENTA PARA O MONITORAMENTO E PERÍCIA AMBIENTAL EM ÁREAS DE MINERAÇÃO

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Tese apresentada ao Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, como parte dos requisitos para a obtenção do título de Doutora em Ecologia, Conservação e Manejo da Vida Silvestre.

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RESUMO

A avaliação ecotoxicológica é um instrumento legal de gerenciamento ambiental, utilizada no Brasil para o monitoramento da qualidade das águas superficiais, a qual pode fornecer subsídios para a perícia ambiental em locais suspeitos de poluição. As atividades minerárias são uma das maiores fontes de contaminação por metais e metaloides, que se acumulam no sedimento em concentrações potencialmente tóxicas para a biota. Entretanto, pouca atenção tem sido dada à ecotoxicidade dos sedimentos e solos, assim como à importância da utilização de espécies nativas no monitoramento ambiental. Neste contexto, foi realizada uma avaliação ecotoxicológica da água superficial, sedimentos e solos em locais no alto curso da bacia do rio São Francisco, MG, utilizando bioindicadores aquáticos padronizados internacionalmente (*Daphnia similis*, *Ceriodaphnia dubia*) e representativos de ambientes tropicais (*Daphnia laevis*, *Ceriodaphnia silvestrii, Chironomus xanthus*). Este estudo visou analisar o monitoramento de águas superficiais vigente no Estado de Minas Gerais e subsidiar alternativas para o monitoramento e perícia ambiental, com ênfase em áreas de mineração. Os resultados mostraram efeitos de toxicidade aguda e crônica da água mesmo em locais onde os elementos químicos quantificados estiveram de acordo com a legislação. Os sedimentos apresentaram elevadas concentrações de metais e arsênio. Não houve toxicidade aguda em *C. xanthus*. Entretanto, verificou-se redução significativa na reprodução de *C. dubia* e *C. silvestrii* expostas aos sedimentos bem como efeitos de toxicidade aguda e crônica aos cladóceros expostos aos elutriatos dos sedimentos. Os experimentos com os elutriatos de solos demonstraram que eles possuem baixa capacidade de retenção, podendo atuar como fontes de contaminantes. Em geral, os efeitos tóxicos foram maiores no período de seca, especialmente nos locais a jusante de atividades minerárias. *D. laevis* se mostrou mais sensível que *D. similis* em locais altamente contaminados por metais. *C. dubia* e *C. silvestrii* não apresentaram diferenças significativas na reprodução entre si quando expostas à água superficial e sedimento total. Entretanto, para os elutriatos de sedimentos e solos houve diferenças sem um padrão definido entre locais e período de amostragem. Nos experimentos com o sedimento da área de beneficiamento de ouro, os testes de fuga com *C. xanthus* não se apresentaram como uma boa ferramenta para avaliação da contaminação de sedimentos por metais. Por outro lado, observou-se toxicidade crônica, caracterizada por menor crescimento das larvas e atraso no tempo de emergência de *C. xanthus* expostos às amostras de sedimento. Verificou-se ainda bioacumulação de metais e arsênio por *C. xanthus*, assim como dos organismos coletados em campo, demonstrando assim, os impactos da atividade minerária sobre os organismos bentônicos. O presente estudo mostrou a importância de se incluir a avaliação ecotoxicológica de sedimentos e solos no monitoramento ambiental, bem como de se adotar organismos nativos nos estudos ecotoxicológicos. A abordagem ecotoxicológica mostrou-se eficiente para utilização como ferramenta na perícia ambiental em áreas de mineração.

PALAVRAS-CHAVE: Ecotoxicologia; mineração; sedimentos; espécies nativas; monitoramento e perícia ambiental.

ABSTRACT

The ecotoxicological assessment is a legal instrument of environmental management used in Brazil for surface water quality monitoring, which can provide subsidies for environmental forensic investigation at sites suspected of pollution. Mining activity is a major source of contamination by metals and metalloids that accumulate in the sediment at high concentrations potentially toxic to biota. However, little attention has been given to sediments and soils ecotoxicity, as well as to the importance of using native species in environmental monitoring. In this context, an ecotoxicological assessment of surface water, sediments and soils at sites in the upper course of the São Francisco river basin, was carried out using internationally standardized aquatic bioindicators (*Daphnia similis*, *Ceriodaphnia dubia*) as well as representatives of tropical environments (*Daphnia laevis*, *Ceriodaphnia silvestrii*, *Chironomus xanthus*). This study aimed to analyze the current surface water monitoring of Minas Gerais State and subsidize alternatives for environmental monitoring and forensic investigation, with emphasis on mining areas. The results showed acute and chronic toxicity effects of water even at sites where chemical elements were in compliance with legislation. The sediments presented high metals and arsenic concentrations. No acute effects in *C. xanthus* were observed. However, there was a significant reduction in reproduction of *C. dubia* and *C. silvestrii* exposed to sediments as well as acute and chronic toxicity to cladocerans exposed to sediment elutriates. Experiments with soil elutriates demonstrated that they have low retention capacity and can act as contaminants sources. In general, the toxicity effects were higher in the dry season, especially at sites downstream of mining activities. *D. laevis* was more sensitive than *D. similis* in sites highly contaminated by metals. *C. dubia* and *C. silvestrii* did not present significant differences in reproduction between themselves in surface water and whole sediment samples. However, for elutriates of sediment and soil, there were differences, without a defined pattern among sites and season. In the experiments with the sediment of the gold processing area, avoidance tests with *C. xanthus* did not show to be a good tool to evaluate sediments contaminated by metals. On the other hand, chronic toxicity was observed, where growth (dry weight) and time of emergency of *C. xanthus* larvae exposed to sediment samples were significantly delayed compared to controls. It was also found bioaccumulation of metals and arsenic in *C. xanthus*, as well as in indigenous organisms, demonstrating the impacts of mining activity on a benthic organism. This study showed the importance of including sediment and soil ecotoxicological assessment in environmental monitoring, as well as to consider native organisms in ecotoxicological assays. These assays also showed to be effective in environmental forensic investigation of mining areas.

KEYWORDS: Ecotoxicology; mining activity; sediments; native species; environmental monitoring; environmental forensic investigation.

SUMÁRIO

1 – INTRODUÇÃO GERAL

1.1 – Abordagem Ecotoxicológica no Monitoramento Ambiental

A Ecotoxicologia é definida como a "ciência que estuda os efeitos das substâncias naturais ou sintéticas sobre os organismos vivos, populações e comunidades, animais ou vegetais, terrestres ou aquáticos, que constituem a biosfera, incluindo assim a interação das substâncias com o meio nos quais os organismos vivem num contexto integrado" (PLAA, 1982, CAIRNS e NIEDERLEHNER, 1995 *apud* ZAGATTO, 2006). Surgiu como ferramenta de monitoramento ambiental, baseada principalmente na resposta de organismos a poluentes naturais ou sintéticos (MAGALHÃES e FERRÃO FILHO, 2008). Assim, a aplicação de testes ecotoxicológicos integra os conceitos da Ecologia – em relação à diversidade e representatividade dos organismos, e da Toxicologia – contemplando os efeitos adversos dos poluentes sobre as comunidades biológicas (PLAA, 1982, *apud* Zagatto, 2006).

Por outro lado, análises físicas e químicas convencionais, utilizadas na avaliação da qualidade de água, sedimento e solo, não são completamente eficientes, pois não indicam as substâncias causadoras de efeitos nos organismos e as inertes no ambiente. É, portanto, necessário que os efeitos causados pelos poluentes sejam avaliados utilizando-se bioindicadores em testes de toxicidade, além da verificação da presença ou ausência de organismos no ambiente. Além disso, com o aumento da diversidade e complexidade das substâncias liberadas no ambiente, a importância dos testes de toxicidade torna-se ainda mais evidente (COSTA *et al*., 2008; MAGALHÃES e FERRÃO FILHO, 2008).

Os métodos disponíveis permitem a observação de efeitos mais drásticos, como mortalidade e imobilidade, caraterizados pelos testes de toxicidade aguda, e também de efeitos subletais, como alterações comportamentais e fisiológicas em prazo mais longo, verificados pelos testes de toxicidade crônica. No ambiente aquático, em geral, os organismos estão expostos a níveis subletais de poluentes, devido a fatores de diluição, sendo necessário o uso dos testes de toxicidade crônica para detecção desses efeitos (ARAGÃO e ARAÚJO, 2006).

No que ainda se refere à avaliação da qualidade de ecossistemas aquáticos, destaca-se a importância dos sedimentos, visto que eles podem apresentar elevados níveis de poluentes, resultantes da disposição de efluentes líquidos e resíduos nos cursos de água ao longo do tempo. Os compostos tóxicos presentes nos ambientes aquáticos podem se depositar no sedimento e se associar a partículas, sofrer transformações, serem absorvidos por organismos bentônicos ou liberados para a coluna d'água (ARAÚJO *et al*., 2006). Embora exista um fluxo contínuo de compostos orgânicos e inorgânicos na interface sedimento-água, os sedimentos possuem características mais permanentes do que a coluna de água, fornecendo um registro histórico das atividades ao redor do ecossistema aquático (BURTON, 1991), podendo ser considerado um reservatório dos poluentes introduzidos nos corpos hídricos superficiais (SIMKISS *et al*., 2001).

A utilização dos testes ecotoxicológicos já se encontra estabelecida na legislação brasileira referente à qualidade das águas superficiais pela Resolução CONAMA n° 357/05 (BRASIL, 2005), como padrão de qualidade de rios. Porém, no Brasil não há legislação específica para qualidade dos sedimentos, sendo geralmente utilizados os níveis de classificação estabelecidos na Resolução CONAMA n° 454/12 (BRASIL, 2012), elaborada para a avaliação de material a ser dragado, como valores orientadores na avaliação dos estudos. Esta Resolução considera a realização de ensaios ecotoxicológicos, porém, condicionados aos resultados das análises químicas, sendo opção do empreendedor a realização de ensaios de toxicidade aguda ou crônica.

Enquanto a ecotoxicologia aquática já se estabeleceu como um instrumento de monitoramento ambiental, os estudos ecotoxicológicos terrestres ainda são recentes e estão se consolidando no Brasil (NIVA *et al*., 2016). O solo possui diversas funções, como habitat para a fauna, flora e os seres humanos, fornecimento de alimento, ciclagem de nutrientes, além de atuar como um sumidouro de compostos orgânicos e inorgânicos perigosos, agindo como um tampão para proteção das águas subterrâneas (SOUSA *et al*., 2008). Assim, a avaliação ecotoxicológica de solos deve considerar as funções a serem protegidas, especialmente as funções de hábitat e de retenção (ISO, 2003). No contexto legal, a Resolução CONAMA nº 420/09 estabelece valores orientadores de qualidade do solo quanto à presença de substâncias químicas e estabelece diretrizes para o gerenciamento ambiental de áreas contaminadas (BRASIL, 2009). Entretanto, nenhuma referência é dada à necessidade de avaliações ecotoxicológicas como ferramenta de análise para qualidade dos solos.

A Associação Brasileira de Normas Técnicas (ABNT) estabeleceu protocolos para condução de ensaios ecotoxicológicos com organismos de água doce de diferentes níveis tróficos. Dentre eles, os que utilizam ensaios de toxicidade aguda com *Daphnia similis* e *Daphnia magna* (ABNT NBR 12713:2016) e de toxicidade crônica com *Ceriodaphnia dubia* e *Ceriodaphnia silvestrii* (ABNT NBR 13373:2017) para amostras de água e ensaios de toxicidade aguda e crônica com *Hyalella azteca* (ABNT NBR 15470:2013) para amostras de sedimento. Em relação a solos, utiliza-se o ensaio de toxicidade aguda para *Eisenia fetida* e/ou *Eisenia andrei* (ABNT NBR 15537:2014). Observa-se que estas normas são baseadas em normas de países de região temperada, desenvolvidas para espécies locais, sendo que das espécies consideradas nestas normas da ABNT, apenas *C. silvestrii* é característica de região tropical.

O mesmo ocorre com os padrões de qualidade estabelecidos para água, sedimento e solo, nas respectivas Resoluções CONAMA. De acordo com Bertoletti e Zagatto (2006), a maioria dos limites de qualidade de água constantes nas legislações de vários países tem origem nos critérios dos países do hemisfério norte, particularmente Estados Unidos e Canadá, sendo adotados como padrões gerais, sem considerar as características ambientais de cada país. Os níveis de classificação adotados na Resolução CONAMA n° 454/12 têm como referência publicações oficiais canadenses, norte-americanas e europeias (BRASIL, 2012), enquanto os valores orientadores da Resolução CONAMA nº 420/09 são baseados em valores holandeses (NIEMEYER *et al.*, 2015). Entretanto, a sensibilidade das espécies de regiões temperadas e tropicais pode ser diferente para algumas substâncias, devido às respostas espécie-específicas e aos fatores ambientais característicos de cada região (KWOK *et al.*, 2007; DAAM e VAN DEN BRINK, 2010; FREITAS e ROCHA, 2012; MOREIRA *et al.*, 2014), o que pode gerar critérios e consequentemente, padrões diferentes.

Neste sentido, pesquisas têm sido conduzidas visando o levantamento de dados científicos relacionados ao cultivo, ciclo de vida e sensibilidade de cladóceros nativos e sua aplicação em estudos ambientais (FONSECA e ROCHA, 2004a; FREITAS e ROCHA, 2011; RIBEIRO, 2011; RIETZLER *et al.*, 2017; MANSANO *et al.*, 2016), assim como de organismos bentônicos (FONSECA e ROCHA, 2004b; ALMEIDA, 2007; SUEITT *et al.*, 2015; RIETZLER *et al.*, 2017). Estes estudos tem enfatizado a melhor representatividade das espécies nativas às condições ambientais das regiões tropicais, em detrimento de espécies de regiões temperadas, além de se evitar o risco de introdução acidental de espécies exóticas.

1.2 – Perícia Ambiental

No Brasil, a criminalização das ações danosas ao meio ambiente foi definida pela Lei n° 9605/1998 (BRASIL, 1998), conhecida como "Lei de Crimes Ambientais", estabelecendo penalidades aos responsáveis. Foram estabelecidos os crimes contra a fauna, a flora, de poluição, contra o ordenamento urbano e o patrimônio cultural e contra a administração ambiental, cabendo aos Órgãos de Segurança Pública o levantamento de provas relativas aos crimes ambientais (BARBIERI *et al.*, 2007; ALVES, 2009). O levantamento de provas é realizado pela perícia ambiental, necessária tanto para comprovar o dano, como para comprovar a possibilidade de prejuízos à saúde humana, à fauna e à flora (BELLO FILHO, 2003; ALVES, 2014).

A partir da verificação de um possível crime de poluição ambiental, o levantamento de dados, realizado pelos peritos, para sua caracterização e comprovação, é uma ferramenta para a avaliação e julgamento do processo. Entretanto, por ser uma área recente da criminalística e pela escassa produção científica na área da perícia ambiental, verifica-se a necessidade de determinação de métodos aplicáveis à caracterização de crimes ambientais pela perícia (ALVES, 2009).

Além disso, existe uma insuficiência de recursos, como laboratórios e equipamentos, para a condução das análises necessárias das provas ambientais que posam levar à evidência do crime de poluição (BARBIERI *et al.*, 2007; BARBIERI, 2015). Deve-se considerar ainda a grande quantidade de substâncias químicas existentes e as poucas informações toxicológicas, dificultando a caracterização e definição da fonte de poluição devido aos custos dos equipamentos, o que torna inviável a caracterização de amostras complexas. Neste contexto, a Ecotoxicologia possui características necessárias às ferramentas de perícia ambiental, como baixo custo e rapidez na obtenção dos resultados, sendo importante destacar a utilização de organismos, os quais são o objeto de conservação, como sensores. Entretanto, poucos estudos foram realizados com este enfoque (ALVES, 2009; 2014).

Dentre os poucos estudos realizados no Brasil utilizando a avaliação ecotoxicológica para elaboração de provas de crime de poluição ambiental, inclui-se o de avaliação de efluentes de curtume, em que foram realizados ensaios de toxicidade aguda e crônica com água superficial e efluentes, utilizando os microcrustáceos *Daphnia laevis*, *Daphnia similis* e *Ceriodaphnia silvestrii*. Foram observados efeitos na sobrevivência e reprodução, os quais estiveram relacionados com altas concentrações de cromo nas amostras (ALVES e RIETZLER, 2014). Na avaliação ecotoxicológica dos sedimentos, verificou-se toxicidade aguda a *Chironomus xanthus* e crônica a *Ceriodaphnia silvestrii*, havendo correlação desses resultados com a tríade de qualidade de sedimentos, que também apresentou baixa biodiversidade da macrofauna bentônica e altas concentrações de cromo (ALVES e RIETZLER, 2015a). O estudo concluiu que a avaliação ecotoxicológica do sedimento pode ser usada com eficácia para fornecer provas de impacto ambiental.

Alves (2014) realizou uma avaliação ecotoxicológica de solos do entorno de duas áreas de mineração em Minas Gerais com o oligoqueto *Eisenia andrei*, utilizando parâmetros letais e subletais como subsídios para a perícia ambiental. Embora não tenham sido observados efeitos tóxicos na sobrevivência e na variação de biomassa, houve diminuição da reprodução, resposta de fuga das amostras de solos, bioconcentração de arsênio nos organismos e genotoxicidade nos organismos expostos às amostras dos dois locais. Todos os efeitos estiveram correlacionados às elevadas concentrações de arsênio nos solos. Dentre todos os parâmetros avaliados, os ensaios de fuga apresentaram a melhor relação benefícios/custos como ferramenta para a perícia ambiental relacionada aos impactos das minerações sobre a fauna edáfica.

Ressalta-se a necessidade do desenvolvimento de pesquisas na área forense ambiental no Brasil, voltados para a aplicação da abordagem ecotoxicológica na perícia ambiental, bem como de parcerias envolvendo a comunidade acadêmica e os órgãos de perícia oficial (ALVES, 2014; BARBIERI, 2015). Estas parcerias seriam de grande importância para ambos os envolvidos, visto que um trabalho interdisciplinar, baseado em métodos científicos de diferentes áreas ambientais, poderia levar à elaboração de provas robustas, à formação de mão de obra capacitada e ao avanço científico.

1.3 – Área de estudo

O rio das Velhas é o maior afluente em extensão do rio São Francisco. A sub-bacia do rio das Velhas está localizada na região do Alto São Francisco, região central do estado de Minas Gerais (Figura 1), onde se destacam as demandas urbana e industrial, principalmente a siderurgia, mineração, química, têxtil, papel e equipamentos industriais, contribuindo para a degradação das águas do rio das Velhas (CBH Rio das Velhas, 2015; CBHSF, 2015). Devido à sua extensão, a bacia é dividida em alto, médio e baixo curso. O alto curso engloba desde sua nascente, no município de Ouro Preto, até a região metropolitana de Belo Horizonte (NONATO *et al*., 2007).

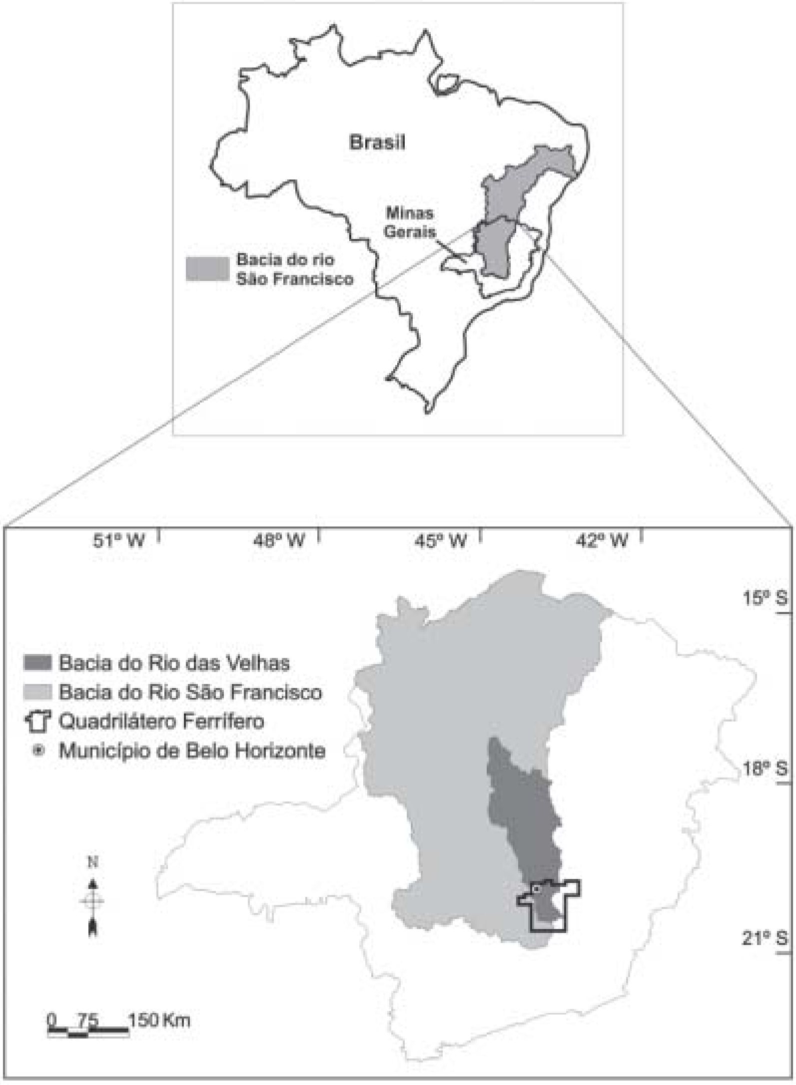

Figura 1 – Localização da área de estudo (SALGADO *et al.*, 2007).

O alto curso da bacia do rio das Velhas é uma região de nascentes e um dos principais mananciais de abastecimento urbano de água da região metropolitana de Belo Horizonte (PEREIRA *et al.*, 2007; NONATO *et al.*, 2007). Possui clima tropical úmido, com invernos secos e verões chuvosos entre os meses de outubro e abril (MORENO *et al.,* 2010; FEIO *et al*., 2015). Sua área está toda inserida na região do Quadrilátero Ferrífero.

O Quadrilátero Ferrífero (QF) é um dos mais importantes depósitos minerais do mundo, conhecido por sua grande produção de minério de ferro, alumínio, topázio e ouro (DESCHAMPS *et al*., 2002). Os principais depósitos de ouro são encontrados nas rochas do grupo Nova Lima, base do Supergrupo Rio das Velhas, associadas principalmente às formações ferríferas de pirita (FeS_2), pirrotita (FeS) e arsenopirita ($FeAsS$) (BORBA *et al*., 2000). Assim, as altas concentrações de metais e arsênio nos solos do QF estão relacionadas à presença de arsênio e sulfetos no minério de ouro primário e à mineralização de ferro (BORBA *et al*., 2000; SOUZA *et al*., 2015).

As concentrações de metais e arsênio quantificadas nos solos do QF estão acima dos valores de referência de qualidade (representam a concentração natural) determinados para Minas Gerais pela Deliberação Normativa Conjunta COPAM/CERH nº 02/2010 (MINAS GERAIS, 2010), caracterizando uma anomalia natural da região (SOUZA *et al*., 2015). Consequentemente, devido à interação entre geologia e interferência humana, concentrações anômalas são também encontradas nos sedimentos (VICQ *et al*., 2015).

Estudos têm sido realizados visando caracterizar as concentrações de metais e arsênio na água, sedimento e solo no QF (BORBA *et al*., 2000; DESCHAMPS *et al*., 2002; PIMENTEL *et al*., 2003; VIGLIO e CUNHA, 2010; VAREJÃO *et al*., 2011; VICQ *et al*., 2015), demonstrando altas concentrações naturais, mas que foram intensificadas pela exploração minerária desenvolvida há mais de trezentos anos, especialmente nas proximidades da região metropolitana de Belo Horizonte (COSTA *et al*., 2015), onde já foram detectadas elevadas concentrações de arsênio em crianças (MATSCHULLAT *et al*., 2000). Durante a época de chuvas, a contaminação por metais, na água e sedimento, gerada no alto curso da bacia atinge uma distância de aproximadamente 400 km, contaminando peixes e áreas agrícolas (VEADO *et al*., 2006).

Entretanto, poucos estudos avaliaram os efeitos tóxicos destas altas concentrações sobre a biota. Sales (2013) avaliou a ecotoxicidade de córregos de duas áreas do QF, uma na bacia do rio das Velhas, influenciada pela atividade de beneficiamento de ouro, e outra na bacia do rio Doce, em área de extração de ouro. Foram verificados efeitos tóxicos a cladóceros tanto na água como no sedimento em ambas as áreas, sendo que a área de extração de ouro apresentou efeitos mais severos.

Alves e Rietzler (2015b) estudaram as mesmas duas áreas abordando a toxicidade do solo, onde foram observados efeitos de toxicidade crônica em *Eisenia andrei* nas duas áreas, correlacionados com as altas concentrações de As no solo. Também foram observadas resposta de fuga das amostras de solos, bioconcentração de arsênio nos organismos e genotoxicidade nos organismos expostos às amostras dos dois locais (ALVES, 2014).

No estado de Minas Gerais, seguindo o disposto a nível federal, a Deliberação Normativa Conjunta COPAM/CERH n° 01/2008 (MINAS GERAIS, 2008) emprega os ensaios ecotoxicológicos como padrões de qualidade da água e de lançamento de efluentes, e seus padrões são os utilizados na avaliação da qualidade das águas superficiais no monitoramento realizado pelo Instituto Mineiro de Gestão das Águas - IGAM, dentro do Projeto Águas de Minas.

Este monitoramento é realizado pelo IGAM desde 1997, sendo realizadas campanhas trimestrais para avaliação de parâmetros que compõem os indicadores: Índice de Qualidade das Águas (IQA), Contaminação por Tóxicos (CT) e Índice de Estado Trófico (IET). A CT é avaliada pela concentração de 13 substâncias tóxicas específicas (arsênio total, bário total, cádmio total, chumbo total, cianeto livre, cobre dissolvido, cromo total, fenóis totais, mercúrio total, nitrito, nitrato, nitrogênio amoniacal total e zinco total). São realizados ensaios de toxicidade crônica da água com o microcrustáceo *Ceriodaphnia dubia* (espécie exótica) desde 2001, contemplando atualmente 194 das 580 estações de monitoramento. Em relação à qualidade dos sedimentos, é utilizado o índice BMWP (Biological Monitoring Working Party Score System), que considera a diversidade de macroinvertebrados bentônicos, realizada anualmente em 38 estações de amostragem na bacia do rio das Velhas a partir de 2012 (IGAM, 2017).

1.3.1 – Pontos de coleta

A seleção dos pontos de amostragem no alto curso da sub-bacia do rio das Velhas considerou os pontos do monitoramento realizado pelo IGAM, que fossem representativos de áreas afetadas por diferentes pressões antrópicas, em particular, a atividade minerária. Os locais estudados (Tabela 1, Figura 2) incluíram os pontos AV060 e BV041, onde não há avaliação ecotoxicológica; AV320 e BV067 com a abordagem ecotoxicológica; além de locais a montante dos pontos AV050 e AV320.

O ponto denominado P3 foi escolhido para referência de campo por ser classificado como Classe 1 pela Deliberação Normativa nº 20/1997 (Minas Gerais, 1997). O ponto P1 foi definido a montante do local de monitoramento do IGAM visando uma melhor caracterização dos efeitos da mineração de ferro local, considerando que o Ribeirão do Silva foi diretamente impactado pelo rompimento da barragem de rejeitos da empresa, ocorrido em 10 de setembro de 2014. Considerou-se o ponto P4 com a finalidade de complementar os estudos previamente conduzidos no local por Sales (2013) e Alves (2014), incluindo um local a montante, P5, a fim de caracterizar os efeitos diretos do beneficiamento de ouro. Os pontos P2 e P6 foram escolhidos a fim de se avaliar a efetividade do monitoramento atual no Estado.

Tabela 1 – Descrição dos pontos de amostragem no alto curso da sub-bacia do rio das Velhas.

Ponto	Descrição	Coordenadas	Principais fatores de poluição
P1: A montante de AV 050	Ribeirão do Silva a montante do Córrego das Almas Classe 2	-20° 15' 56,4" -43° 56' 10,0"	Mineração de ferro
P2: AV060	Ribeirão Carioca a montante do ribeirão Mata Porcos Classe 2	-20° 17' 21,9" -43° 48' 18,5"	Erosão
P3 (Referência de campo): BV041	Ribeirão Cortesia a montante de Rio Acima Classe 1	-20° 6' 7" -43° 59' 48"	Pecuária
P4: AV320	Córrego da Mina a montante do rio das Velhas Classe 2	-19° 58' 45,1" -43° 49' 15,2"	Beneficiamento de minério de ouro
P5: A montante de AV 320	Córrego da Mina a montante do rio das Velhas Classe 2	-19° 58' 29,1" -43° 49' 41,5"	Beneficiamento de minério de ouro
P6: BV067	Rio das Velhas a montante do ribeirão Sabará Classe 2	-19° 56' 18,3" -43° 49' 37,7"	Esgotos domésticos, Siderurgia, Metalurgia do ouro.

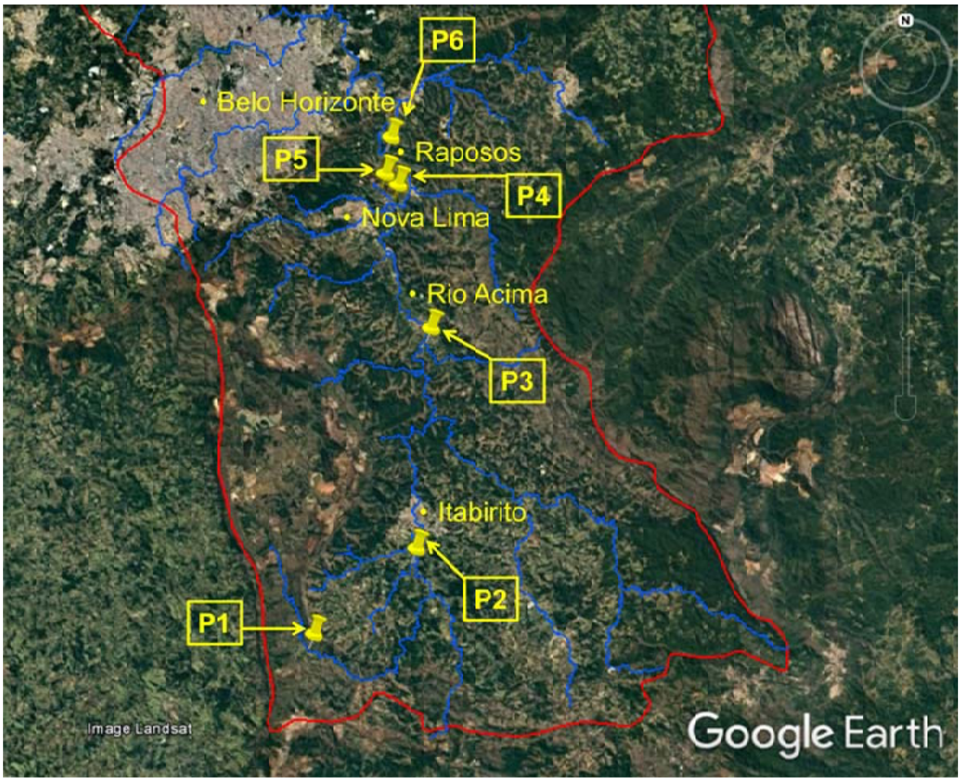

Figura 2 – Localização dos pontos de amostragem na sub-bacia do rio das Velhas.

2 – OBJETIVOS

2.1 – Objetivo Geral:

Avaliar, através de estudos ecotoxicológicos, alternativas para o monitoramento da qualidade de cursos de água de Minas Gerais, como subsídios para a perícia ambiental.

2.2 – Objetivos Específicos:

1 – Realizar testes de toxicidade com amostras de água superficial, sedimentos e solos na bacia do alto São Francisco, em pontos no alto curso da bacia do rio das Velhas com influências antrópicas distintas, utilizando bioindicadores aquáticos padronizados internacionalmente (*Daphnia similis*, *Ceriodaphnia dubia*) e representativos de ambientes tropicais (*Daphnia laevis*, *Ceriodaphnia silvestrii, Chironomus xanthus*), visando avaliar as ferramentas utilizadas no monitoramento ambiental, como subsídios para a perícia ambiental.

2 – Avaliar efeitos tóxicos dos sedimentos de uma área de processamento de ouro sobre *Chironomus xanthus*, considerando o ciclo de vida parcial (crescimento e emergência), o comportamento de fuga e a bioacumulação de metais e arsênio, assim como o uso deste organismo para o monitoramento e perícia ambiental.

3 – JUSTIFICATIVA E HIPÓTESE

Este trabalho foi desenvolvido a partir da ponderação sobre os parâmetros utilizados no monitoramento para avaliação da qualidade das águas superficiais de Minas Gerais e o seu aproveitamento para os trabalhos de perícia ambiental.

O organismo utilizado na avaliação ecotoxicológica, *Ceriodaphnia dubia*, é uma espécie exótica, característica de países do hemisfério norte, aclimatada para as condições tropicas, não sendo, assim, a mais indicada para utilização. Além disso, a espécie nativa *Ceriodaphnia silvestrii*, já está padronizada pela ABNT, e possibilita respostas mais representativas dos ambientes estudados. Não há, portanto, justificativa para a utilização da espécie exótica, apenas, no programa de monitoramento do estado.

Por outro lado, não há monitoramento da qualidade dos sedimentos no estado, sendo que a única abordagem existente é a avaliação da diversidade de macroinvertebrados bentônicos em poucos pontos de amostragem na bacia do rio das Velhas. Tendo em vista que os sedimentos atuam como sumidouro e fonte de contaminantes, muitas vezes em pequenas concentrações ou não detectados na coluna d'água, verifica-se a necessidade de se incluir os sedimentos no monitoramento dos cursos de água, considerando não apenas a quantificação de substâncias químicas, mas também seus efeitos ecotoxicológicos.

Neste sentido, considera-se a hipótese de que os parâmetros utilizados no monitoramento da qualidade das águas superficiais no estado de Minas Gerais não são suficientes para determinar a qualidade ambiental dos cursos d'água, verificando-se a necessidade de desenvolvimento de estudos que possam propor ferramentas mais adequadas para a avaliação da qualidade de ambientes aquáticos e que possam ser utilizadas para a construção de provas pela perícia ambiental em locais suspeitos de poluição.

A seleção dos pontos de amostragem no alto curso da bacia do Rio das Velhas levou em consideração os pontos do monitoramento realizado pelo órgão ambiental e que fossem representativos de áreas afetadas por diferentes pressões antrópicas, enfatizando atividades minerárias. Os locais estudados incluem pontos que fazem parte da rede de monitoramento do IGAM, contemplando ou não a avaliação ecotoxicológica, além de locais a montante de pontos de monitoramento. O ponto escolhido para referência de campo é classificado como Classe 1. Considerando que também não há monitoramento da qualidade dos solos, realizou-se a avaliação da função de retenção dos solos das proximidades dos cursos de água, a fim de verificar o potencial dos solos como fonte de contaminação das águas subterrâneas, além de avaliar a possibilidade da utilização dos cladóceros nativos para esta abordagem ecotoxicológica.

O presente trabalho faz parte de uma proposta aprovada nos termos do Edital Programa Ciências Forenses nº 25/2014, pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES.

Esta tese está estruturada em dois capítulos em formato de artigos, descritos a seguir:

- Capítulo 1: "Ecotoxicological approach in environmental monitoring of the Iron Quadrangle, Minas Gerais, Brazil".

- Capítulo 2: "Chironomids as indicators of contamination in gold processing areas: sublethal effects of sediments on *Chironomus xanthus*".

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5 - CAPÍTULO 1: Ecotoxicological approach in environmental monitoring of the Iron Quadrangle, Minas Gerais, Brazil.

Abstract

The ecotoxicological assessment is an instrument of environmental management established in Brazilian environmental legislation for surface water quality monitoring. However, little attention has been given to sediments and soils ecotoxicity, as well as to the importance of using native species in environmental monitoring. In this context, an ecotoxicological assessment of surface water, sediments and soils at sites in the upper course of the São Francisco river basin, was carried out using internationally standardized aquatic bioindicators (*Daphnia similis*, *Ceriodaphnia dubia*) as well as representatives of tropical environments (*Daphnia laevis*, *Ceriodaphnia silvestrii*, *Chironomus xanthus*), aiming to analyze the current surface water monitoring of Minas Gerais State and subsidize alternatives for environmental monitoring and forensic investigation, with emphasis on mining areas. The results showed acute and chronic toxicity effects of water even at sites where chemical elements were in compliance with legislation. The sediments presented high metals and arsenic concentrations. However, no acute effects in *C. xanthus* were observed. On the other hand, there was chronic toxicity effects in *C. dubia* and *C. silvestrii* exposed to sediments, even in places without water toxicity. Assays with elutriate sediments showed that it is possible to evaluate the toxic effects of sediment resuspension using this sample phase. Experiments with soil elutriates demonstrated that they have low retention capacity and can act as contaminants sources for ground and surface waters. *D. laevis* was more sensitive than *D. similis* in sites highly contaminated by metals, in water and in elutriates of sediment and soil. *C. dubia* and *C. silvestrii* did not present significant differences in reproduction between themselves in surface water and whole sediment samples. However, for elutriates of sediment and soil, there were differences, without a defined pattern among sites and season. Thus, this study showed the importance of including sediment and soil ecotoxicological assessment in environmental monitoring, as well as to consider native organisms in ecotoxicological assays. These tests also showed to be effective in environmental forensic investigation of mining areas.

Keywords: Ecotoxicology; mining; surface waters; sediments; soil retention function; native species.

1 – Introduction

Ecotoxicology, as an environmental monitoring tool, is based mainly on the organism's response to natural or synthetic pollutants (MAGALHÃES and FERRÃO FILHO, 2008). It contributes to chemical analyses traditionally used in quantification of isolated substances, which are not able to detect possible interactions between these substances (synergistic, additive, antagonistic), and also do not show its bioavailability and effects on biota (COSTA *et al*., 2008).

From the ecotoxicological point of view, the water, sediment and soil quality standards, established in the Brazilian environmental legislation are adapted from northern hemisphere countries, not considering regional biotic and abiotic characteristics (BERTOLETTI and ZAGATTO, 2006; NIEMEYER *et al*., 2015). Toxicity tests with cladocerans, standardized by the Brazilian Association of Technical Standards (ABNT) and performed in accredited laboratories, use mainly the exotic organisms *Daphnia similis* and *Ceriodaphnia dubia*, although *Ceriodaphnia silvestrii*, a native species, was standardized in 2003.

Currently, ecotoxicological evaluation as an instrument of environmental management is carried out by environmental agencies of several Brazilian states. In Minas Gerais, the monitoring of surface water quality is carried out by the Instituto Mineiro de Gestão das Águas (IGAM), in quarterly campaigns, to evaluate parameters that compose the following indicators: Water Quality Index (IQA), Contamination by Toxic substances (CT) and Trophic State Index (IET). Water chronic toxicity tests with *Ceriodaphnia dubia*, started in 2001, currently comprising 194 of the 580 monitoring stations. The only approach to sediment quality evaluation is the determination of the BMWP index (Biological Monitoring Working Party Score System), which considers the diversity of benthic macroinvertebrates, performed annually from 2012, at 38 sampling stations in the das Velhas river basin, a sub-basin of the São Francisco river basin (IGAM, 2017a).

It is important to emphasize that watercourse monitoring should not be limited to water analysis. Sediments, as an important compartment of water ecosystems, are extremely important in the assessment of aquatic ecosystems contamination due to its ability to accumulate pollutants, which will persist in the environment and may generate toxic effects on aquatic biota (ARAÚJO *et al*., 2006). Although there is a continuous flow of organic and inorganic compounds at the sediment-water interface, sediments have more stable characteristics than the water column, providing a historical record of activities around the aquatic environment (BURTON, 1991).

The sediment toxicity assessment can be performed with different test phases, including whole sediment, elutriate and pore water (BURTON, 1991; ARAÚJO *et al.*, 2006). In environmental monitoring programs, tests with whole sediment can be conducted to identify toxic sites, enabling future investigations (INGERSOLL, 1995). Elutriate is an aqueous solution obtained after addition of dilution water to a solid sample, stirring and decantation (ABNT, 2015). It was developed to simulate the desorption of sediment constituents induced by dredging and its temporal and spatial effects on water quality and biota (BURTON, 1991). Since lotic systems are subject to strong rainwater flows and dredging, which may lead to the resuspension of contaminants, toxicity tests with sediment elutriate are an important tool for environmental risk monitoring and assessment.

Several organisms have been used in sediment toxicity tests, including cladocerans such as *Ceriodaphnia dubia* and *Daphnia magna* (INGERSOLL, 1995; KALINOWSKI and ZAŁĘSKA-RADZIWIŁŁ, 2011). Although being planktonic organisms, daphniids are non-selective filtering agents and can feed on the sediment surface, ingesting both suspended and deposited particles, acting as epibenthic species (BURTON, 1992).

With respect to soils, ecotoxicological assessment should consider the soil functions to be protected, especially retention and habitat functions. The retention function is the ability of soils to adsorb pollutants, preventing them to be dissolved in water and become available to the food web. Its evaluation is done by conducting toxicity tests with soil elutriate, serving as an initial indicator of contamination of interstitial and ground water (ISO, 2003). Toxicity tests with different aquatic organisms (*Daphnia magna, Pseudokirchneriella subcapitata, Vibrio fischeri*, among others) have been used to evaluate the retention function, demonstrating the effectiveness of this approach for soil contamination assessment (ALVARENGA *et al*., 2008; NIEMEYER *et al*., 2015).

Following those considerations, the present study aimed to carry out an ecotoxicological assessment of water, sediments and soils in the upper São Francisco basin at sites on the upper course of das Velhas river sub-basin under different anthropogenic pressures, with emphasis on mining. These sites are located within the Iron Quadrangle region, characterized by high metals concentrations in water, sediment and soil intensified by mining activity (BORBA *et al.*, 2000; NONATO *et al.*, 2007; VIGLIO and CUNHA, 2010; VICQ *et al.*, 2015), which has shown toxic effects of water, sediments and soils (SALES, 2013; ALVES and RIETZLER, 2015a).

For that, internationally standardized aquatic bioindicators (*Daphnia similis, Ceriodaphnia dubia*) and representatives of tropical environments (*Daphnia laevis, Ceriodaphnia silvestrii, Chironomus xanthus*) were used in order to analyze the current surface water monitoring of Minas Gerais State and subsidize alternatives for environmental monitoring and forensic investigation.

2 – Material and Methods

2.1 - Field Samplings

The selection of the sampling sites in the upper course of the São Francisco basin, Velhas river sub-basin, considered areas affected by anthropic pressures, with emphasis on mining activity. The sites (Table 1, Figure 1) included sampling sites of the IGAM monitoring network, two of which, AV060 (here named P2) and BV041 (P3) do not present ecotoxicological evaluation; two sites, AV320 (P4) and BV067 (P6), that present such information; and two sites upstream of their monitoring sites, AV050 (without ecotoxicological evaluation - P1) and AV320 (P5). Sampling sites were chosen based on preliminary ecotoxicological evaluation of water and sediments. The site P3 was chosen as field reference for being classified as Class 1 by the Minas Gerais State Regulatory Deliberation No. 20/1997 (MINAS GERAIS, 1997).

Field sampling was conducted in two rainy and two dry seasons, between 2015 and 2017. Five samplings were performed in the dry season (June, September and October/2015, July and August/2016), and four in the rainy season (February, March and November/2016, and February/2017). Samples were collected in the morning period.

At each sampling site, 2 liters of surface water and 1 kg of sediment, from the banks in backwater areas, were collected. Approximately 1 kg of soil samples were collected in the vicinity of the streams, outside the flood area. All samples were stored in plastic polyethylene bottles, kept on ice and transported to the laboratory.

Table 1 - Description of sampling sites in the upper São Francisco basin, Velhas river sub-basin.

Sampling site	Description	Coordinates	Main pollution factors
P1 (upstream of AV050)	do Silva stream, upstream of das Almas stream Class 2	-20° 15' 56,4" -43° 56' 10,0"	Iron Mining
P2 (AV060)	Carioca stream, upstream of Mata Porcos stream Class 2	-20° 17' 21,9" -43° 48' 18,5"	Erosion
P3 (field reference-BV041)	Cortesia stream, upstream of Rio Acima Class 1	-20° 6' 7" -43° 59' 48"	Livestock
P4 (AV320)	da Mina stream, upstream of das Velhas river Class 2	-19° 58' 45,1" -43° 49' 15,2"	Processing of gold ore
P5 (upstream of AV320)	da Mina stream, upstream of das Velhas river Class 2	-19° 58' 29,1" -43° 49' 41,5"	Processing of gold ore
P6 (BV067)	das Velhas river upstream of Sabará stream Class 2	-19° 56' 18,3" -43° 49' 37,7"	Domestic sewage, steel, gold metallurgy

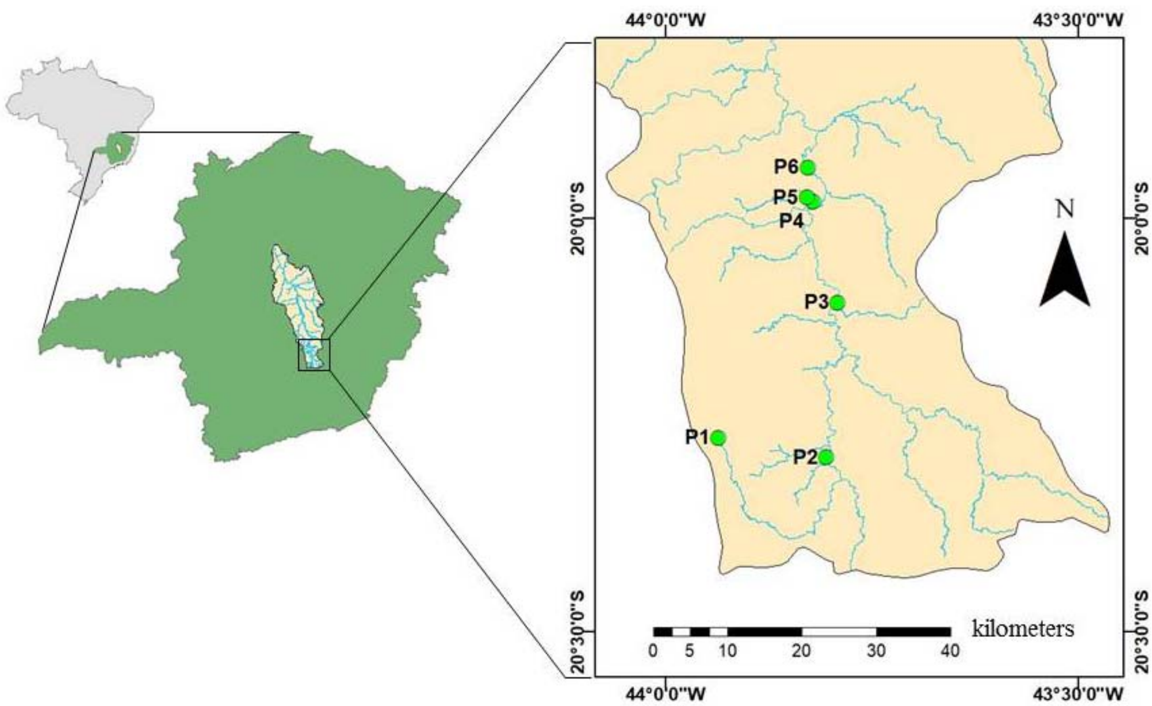


Figure 1 - Location of sampling sites in the upper São Francisco basin, das Velhas river sub-basin.

2.2 - Laboratory Procedures

The water and sediment samples were kept at 4 °C, according to NBR 15469 (ABNT, 2015). Approximately 100 ml of water samples were immediately filtered and acidified with ultra-pure nitric acid, for further quantification of dissolved metals. The parameters pH, dissolved oxygen, conductivity and hardness of the water were measured. The soil samples were kept at room temperature for drying.

Granulometric analysis and the organic matter content of sediments and soils were carried out following EMBRAPA (1997). The quantification of dissolved metals and metalloid in the water samples was performed by inductively coupled plasma mass spectrometry (ICP-MS Perkin Elmer ELAN DRC-e). Determination of metals and metalloid in sediments and soils followed Method 3051A (USEPA, 2007) for digestion with nitric acid in microwave (MARS-CEM). Metal quantification was performed by inductively coupled plasma optical emission spectrometry (ICP-OES Spectro Spectroflame 4165/91).

In order to evaluate the soil retention function, the prevention values (PV), established by the Minas Gerais State Joint Regulatory Deliberation COPAM/CERH No. 02/2010 (MINAS GERAIS, 2010), were used for comparison with the metals concentrations obtained in the soils samples. According to this Deliberation, PV is the concentration of a certain substance above which changes in soil quality may occur in relation to its main functions.

2.2.1 - Test Organisms culturing

The test organisms were maintained in the Laboratory of Ecotoxicology, Institute of Biological Sciences, Federal University of Minas Gerais. *Daphnia similis* was kept according to NBR 12713 (ABNT, 2009); *Ceriodaphnia silvestrii* and *Ceriodaphnia dubia* according to NBR 13373 (ABNT, 2005); and *Daphnia laevis* with adaptations of NBR 12713 (ABNT, 2009). *Chironomus xanthus* were cultured based on Fonseca and Rocha (2004a). The culturing conditions are described in Table 2.

Table 2 - Culturing conditions of the test organisms.

Culturing conditions	*D. similis*	*D. laevis*	*C. dubia* *C. silvestrii*	*C. xanthus*
Culturing water	Natural Source: neutral pH; Hardness: 45-55 mg$CaCO_3$.L^{-1}; Dissolved oxygen: 6-7 mg.L^{-1}; Electric conductivity: 160-180 µS.cm^{-1}			
Number of organisms/container	25	30	70	100
Container	Becker 1 L			Plastic tray with screen for adult retention. 1:4 calcined sand and water.
Water exchange	3 times a week	2 times a week	3 times a week	No
Type and amount of food per day	$2x10^5$cel.ml^{-1} *Raphidocelis subcapitata*	$2x10^5$cel.ml^{-1} *R. subcapitata* + *Kirchneriella obesa*; 75µl Peat Extract	$2x10^5$cel.ml^{-1} *R. subcapitata*; 1ml RL (fermented fish feed and yeast)	0.04 mg.ml^{-1} fish feed; $2x10^5$cel.ml^{-1} *R. subcapitata* only on the 1st day
Aeration	No			Yes
Temperature	20 ± 2°C	25 ± 2°C		
Photoperiod	12 h			
Duration of cultures	4 weeks	3 weeks	3 weeks	2 weeks
Sensitivity control	Monthly test with NaCl			Monthly test with KCl

2.2.2 - Preparation of sediment and soil elutriates

Samples were prepared on the day of the acute toxicity tests and of samples renewal in the chronic toxicity tests, based on NBR 15469 (ABNT, 2015). The elutriates were obtained by mixing one part of the sediment/soil and four of culturing water. The mixtures were kept under stirring during 30 minutes for sediments and 20 hours for soils. After decantation the supernatants were transferred to 100 ml Erlenmeyer, avoiding resuspension.

2.2.3 - Toxicity assays

Acute and chronic toxicity tests were performed with water, sediment and soil samples and respective organisms as described in Table 3. Tests with surface water were started on the same day of sampling. Whenever necessary, tests and laboratory controls used culturing water. For the surface water and elutriate samples, acute and chronic toxicity tests were always conducted in order to verify possible differences in responses between the cladocerans tested.

Table 3 - Description of acute and chronic toxicity tests with surface water, sediment and soil elutriates and whole sediment.

Test conditions	Acute Toxicity Tests		Chronic Toxicity Tests
Test organism	*D. similis* *D. laevis*	*C. xanthus*	*C. dubia* *C. silvestrii*
Protocol	NBR 12713 (ABNT, 2009)	FONSECA (1997)	NBR 13373 (ABNT, 2005)
Sample	Surface water Sediment elutriate Soil elutriate	Whole sediment	Surface water Sediment elutriate Soil elutriate Whole sediment
Test system	Static No aeration		Semi Static No aeration
Duration	48 h	96 h	7 days or 3rd brood
Sample volume	10 ml	5 g of sediment 20 ml of water	15 ml (aqueous samples); 5 g of sediment and 20 ml of water
Sample renewal	No	No	Every 2 days. For sediment, only water is exchanged
Feed	No	only on the 1st day	In sample renewal
Replication	4	10	10
Organisms per replicate	5	1	1
Age of organisms	6 a 24 h	3rd instar	24 h
Photoperiod	Dark		12 h
Temperature	25 ± 2°C		
Effect evaluation	Immobility and mortality	Mortality	Reproduction and survival
Expression of results	Qualitative: Toxic or non-toxic effect		
Test Acceptance Criteria	> 90% survival of control organisms		> 80% survival of control organisms > 15 neonates/control organism

2.2.4 - Statistical analysis

To conduct the statistical analysis, the results obtained in each campaign were grouped as dry (June, September and October/2015, July and August/2016) and rainy (February, March and November/2016, and February/2017) seasons.

To test the differences in average rates of mortality among sites, species and seasons, a logistic regression model was fitted with these three explanatory variables (including the interaction between variables). In order to test differences in the average numbers of neonates produced, a generalized linear regression model was fitted with the Poisson distribution, with the same three explanatory variables of the logistic regression. For both analyses, the Tukey's correction was used in the pair-wise multiple comparisons. Statistical analyses were performed using software R version 3.4.2 (R Core Team, 2017).

3 – Results

3.1 - Samples characterization

The quantification of bioavailable metals and metalloid in water samples (Table 4) showed high concentrations of As, Cu, Ni, Zn, Fe and Mn in P4 and P5, exceeding the limits of the Joint Regulatory Deliberation (RD) COPAM/CERH No. 01/2008 (MINAS GERAIS, 2008). It is important to note that this RD considers the total concentration for As, Ni, Zn and Mn. In the present study, the dissolved fractions were much higher than those stablished in the RD, demonstrating that these are critical sites for metal contamination.

As shown in Table 5, sediment samples from all sites presented As concentrations above level 2 of CONAMA Resolution No. 454/12 (BRASIL, 2012). P3 and P6 also showed Cr and Ni concentrations above level 2. As for water, P4 and P5 were the sites that presented all the elements extrapolating levels 1 or 2, with the highest concentrations during the dry periods.

Table 4 – Mean concentrations of dissolved metals and arsenic (μg.L^{-1}), pH, dissolved oxygen (D.O. mg.L^{-1}), electrical conductivity (Cond. μS.cm^{-1}) and hardness (mgCaCO$_3$.L^{-1}) in surface water samples from the sampling sites in the dry and rainy seasons and limits (μg.L^{-1}) established by the Joint Regulatory Deliberation COPAM/CERH No. 01/2008 (MINAS GERAIS, 2008) for rivers class 1 and 2.

	P1		P2		P3		P4		P5		P6		Limits
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	
As	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	**37.95**	**48.40**	**47.95**	**74.50**	6.95	2.01	10 (T)
Cd	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	1 (T)
Pb	0.71	0.47	0.44	0.42	0.39	0.48	0.52	0.48	0.29	0.27	0.45	0.44	10 (T)
Cu	0.68	0.71	0.35	0.98	0.63	1.79	**1087.50**	**25.40**	**1278.50**	**39.03**	2.73	0.83	9 (D)
Cr	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	1.95	< 1.00	2.87	< 1.00	< 1.00	50 (T)
Ni	< 0.30	< 0.3	< 0.3	< 0.3	0.33	0.69	**193.55**	**79.30**	**179.60**	**84.23**	1.55	1.17	25 (T)
Zn	105.50	43.23	54.40	38.27	70.80	65.63	**227.00**	154.20	**232.00**	159.70	57.50	64.50	180 (T)
Al	7.55	13.85	8.53	17.84	8.45	22.11	61.45	64.40	94.50	79.50	7.80	30.40	100 (D)
Fe	56.25	63.40	**310.50**	230.63	154.50	159.13	**2515.50**	**1873.33**	**3070.50**	**1907.00**	155.50	116.73	300 (D)
Mn	8.37	32.33	33.70	62.03	13.33	42.00	**1199.00**	**1108.67**	**969.50**	**1093.00**	**198.50**	**314.00**	100 (T)
Mg	4878.50	2989.00	509.50	402.33	1526.00	1400.00	71950.00	54826.00	72844.50	56969.67	4484.50	2325.33	--
pH	7.90	7.81	7.36	7.39	7.50	7.34	7.22	7.57	7.46	7.68	7.36	7.57	6 – 9
D.O.	7.93	7.08	8.15	7.36	7.97	7.27	7.99	6.29	8.30	6.90	8.10	6.69	> 6.00
Cond.	99.25	103.78	47.13	97.65	96.90	51.05	2279.75	2165.50	2313.75	2263.50	191.80	138.40	--
Hardness	28.00	29.00	6.00	7.00	12.00	9.00	1115.00	1600.00	1255.00	1670.00	60.00	28.00	--

Highlighted concentrations that exceeded the limits. T: Total; D: Dissolved.

Table 5 – Mean concentrations of metals and arsenic ($\mu g.g^{-1}$) in sediment samples from the sampling sites in the dry and rainy seasons and limits established by CONAMA Resolution No. 454/12 (BRASIL, 2012) for levels 1 and 2 ($\mu g.g^{-1}$).

	P1		P2		P3		P4		P5		P6		Levels	
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	1*	2**
As	**13.45**	**142.44**	**17.99**	**46.78**	**22.68**	**68.45**	**6206.65**	**547.75**	**15553.80**	**3500.77**	**127.34**	**78.20**	5.90	17.00
Cd	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	**67.52**	**3.96**	**151.77**	**49.48**	< 0.003	< 0.003	0.60	3.50
Pb	15.34	23.22	30.52	33.23	15.85	16.97	**45.13**	14.22	**55.58**	16.90	26.80	17.92	35.00	91.30
Cu	10.72	20.38	6.04	10.98	14.56	22.52	**512.48**	**282.16**	**519.52**	**320.63**	**44.68**	28.65	35.70	197.00
Cr	16.78	23.68	**53.72**	**74.38**	**138.25**	**169.65**	**172.87**	**81.96**	**108.61**	**71.78**	**109.56**	**147.55**	37.30	90.00
Ni	12.36	**20.46**	6.43	11.78	**42.34**	**54.15**	**86.28**	**37.18**	**77.50**	**51.00**	**47.63**	**39.28**	18.00	35.90
Zn	30.20	43.35	21.78	12.33	47.99	51.29	**333.28**	79.56	**396.11**	**126.61**	**127.48**	43.60	123.00	315.00
Al	9400.00	12411.73	20996.37	19722.66	10116.08	10415.39	16124.58	6083.33	10668.27	5193.30	14504.90	9640.00	--	--
Fe	73550.00	79832.69	35850.10	37973.13	47429.41	49601.76	107763.60	67892.16	112355.77	95003.77	97849.02	78400.00	--	--
Mn	7030.00	8636.35	154.33	253.99	300.18	443.45	1399.23	598.04	1039.42	1491.86	4105.30	1551.50	--	--
Mg	214.35	276.43	455.36	413.28	1687.41	2748.80	3660.28	815.20	4552.88	5587.22	731.05	565.30	--	--

Highlighted concentrations that exceeded the limits.
*Level 1: threshold, below which there is low probability of adverse effects to biota.
**Level 2: threshold, above which there is high probability of adverse effects to biota.

The sediments of P1 and P3 showed higher percentage of coarse sand, P2, P4 and P6 of fine sand, while P5 had a higher percentage of silt (Table 6). In general, all sediments presented low organic matter content, characteristic of mineral sediments (<10%) (UNGEMACH, 1960); the exception was P6, which receives large influence of domestic sewage. Soils presented higher silt content, followed by sand. Soils from P1, P3, P4 and P5 presented high organic matter content, consistent with organic soils (>20%) (EMBRAPA, 1999).

Table 6 - Granulometry and organic matter (OM) of sediment and soil samples.

	Sampling site	Granulometric fractions (%)				OM (%)
		Coarse sand	Fine sand	Silt	Clay	
Sediments	P1	55.80	16.10	24.40	3.70	0.88
	P2	36.30	48.70	9.70	5.20	3.23
	P3	87.60	4.00	6.10	2.30	2.35
	P4	6.60	46.50	38.90	8.00	6.46
	P5	8.04	37.65	45.90	8.42	7.83
	P6	4.20	49.50	38.90	7.40	11.44
Soils	P1	9.00	8.40	70.50	12.10	23.08
	P2	2.50	37.40	45.80	14.20	13.40
	P3	11.90	21.10	52.70	14.20	23.48
	P4	12.50	28.00	43.80	15.70	24.36
	P5	18.60	11.50	51.40	18.40	20.54
	P6	1.00	46.60	45.40	7.00	6.94

Table 7 shows the metals and metalloid concentrations obtained for the soil samples. It was verified that As had concentrations above the PV at all sampling sites. Cr and Ni have exceeded the PVs at P3, P4, P5 and P6.

Table 7 – Mean concentrations of metals and arsenic (μg.g^{-1}) in soil samples from the sampling sites in the dry and rainy seasons and prevention values (PV - μg.g^{-1}) established by Joint Regulatory Deliberation COPAM/CERH No. 02/2010 (MINAS GERAIS, 2010).

	P1		P2		P3		P4		P5		P6		PV
	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	
As	**23.63**	**28.46**	**16.83**	**15.98**	**24.62**	**27.21**	**35.10**	**220.38**	**65.90**	**85.96**	**42.79**	**115.19**	15.00
Cd	<0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	< 0.003	1.30
Pb	12.64	31.89	20.64	38.95	10.31	16.08	5.74	13.09	6.11	10.77	17.43	14.74	72.00
Cu	2.61	12.70	3.55	6.38	12.06	29.91	11.11	35.63	19.10	53.56	24.22	28.57	60.00
Cr	5.34	33.13	30.03	60.20	61.25	**84.71**	**81.30**	**132.21**	**83.40**	**107.50**	**117.21**	52.50	75.00
Ni	<0,013	8.63	4.79	8.32	25.90	**32.48**	14.30	**30.52**	**41.45**	**48.85**	**34.63**	21.93	30.00
Zn	20.08	46.33	23.80	10.64	32.59	28.87	9.02	27.16	3.03	9.75	40.36	28.37	300.00
Al	5098.04	18596.15	14240.38	28627.45	8317.31	8394.23	4626.00	7115.38	10370.00	7384.62	9317.31	5028.85	--
Fe	105490.20	170096.15	22413.46	36960.78	29346.15	29000.00	43370.00	58365.38	48690.00	46288.46	72307.69	57307.69	--
Mn	7441.18	141.15	222.69	251.47	295.58	248.08	275.90	465.67	216.20	355.00	2221.15	1582.69	--
Mg	138.43	256.15	572.12	457.94	1452.88	998.08	486.00	1673.08	154.40	396.06	659.62	402.98	--

Highlighted concentrations that exceeded the limits.

3.2 - Surface water toxicity tests

3.2.1 - Acute toxicity tests

Samples from P4, P5 and P6 caused acute toxicity to *D. laevis*, throughout the evaluated period (Figure 2A). For *D. similis*, samples of P1, P3, P4, P5 and P6 caused acute toxicity in the dry season and only from P4 and P5 in the rainy season.

D. laevis was more sensitive than *D. similis* in P4 and P5, the sites with higher toxicity (Fig. 2B). For both species, P5 was the site of highest toxicity.

(A)

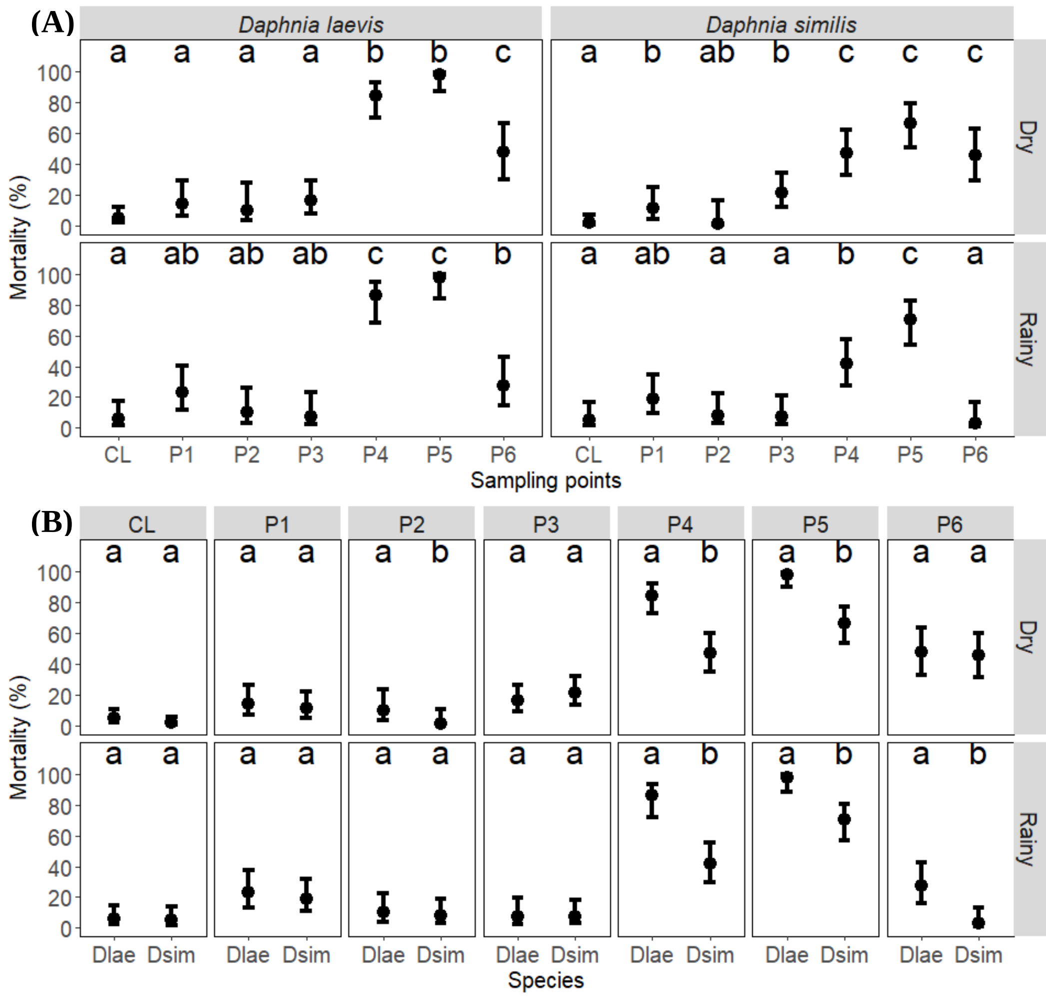


Figure 2 - Mean mortality rate in acute toxicity tests with surface water from the different sampling sites, conducted in the dry and rainy seasons (A) and comparison between the species used in the two periods (B). CL: laboratory control; Dlae: *Daphnia laevis*; Dsim: *Daphnia similis*. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

3.2.2 - Chronic toxicity tests

Samples from P4 and P5 caused a predominant mortality effect for *C. dubia* and *C. silvestrii* (Figure 3), within 48 hours in most assays, characterizing acute toxicity effect. There were no significant differences between species. Toxicity was higher in the dry season.

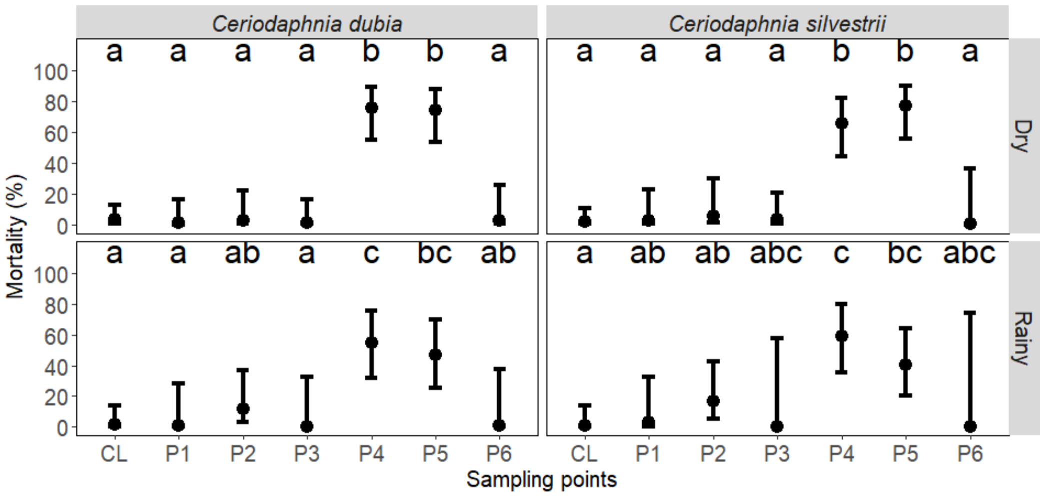


Figure 3 - Mean mortality rate in chronic toxicity tests with surface water from the different sampling sites conducted in the dry and rainy seasons. CL: laboratory control. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

There was no mortality in P4 and P5 in only two campaigns, one in the dry season and one in the rainy, which allowed the evaluation of the reproduction data of these sites.

Samples of P1, P2 and P5 caused chronic toxicity to *C. dubia* in the dry season (Figure 4A). In the rainy season, there was toxic effect of P1, P2, P4, P5 and P6. For *C. silvestrii*, P1, P2 and P4 caused chronic toxicity in the dry season, while P2, P4, P5 and P6 caused toxicity in the rainy period.

In general, *C. dubia* and *C. silvestrii* presented similar reproduction (Fig. 4B), with significant differences only in P4 and P5, in the dry period. For both species, field control (P3) showed similar response to laboratory control, P6 caused toxicity only in the rainy season, while P4 and P5 were the sites of higher toxicity effects.

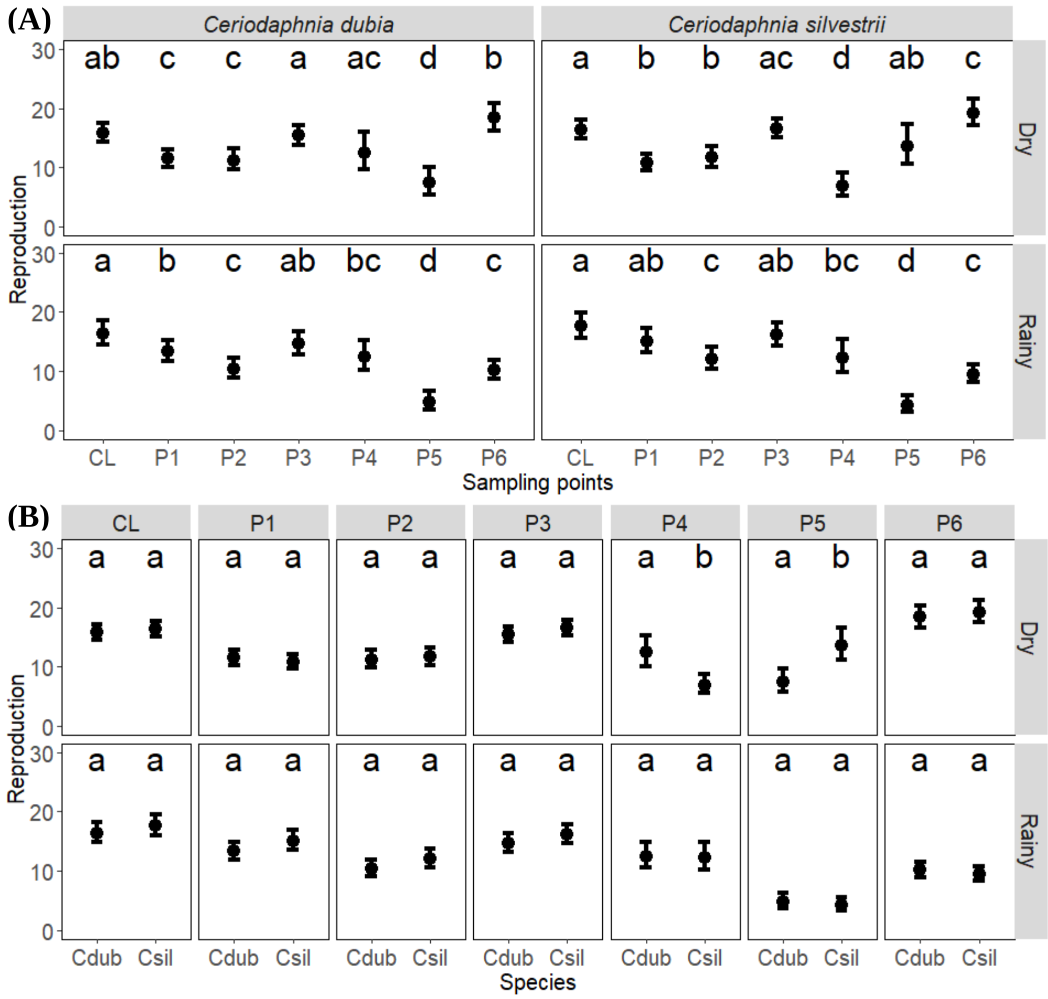


Figure 4 - Mean number of neonates produced in chronic toxicity tests with surface water from the different sampling sites, conducted in the dry and rainy seasons (A) and comparison between the species used in the two periods (B). CL: laboratory control; Cdub: *Ceriodaphnia dubia*; Csil: *Ceriodaphnia silvestrii*. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

In summary, surface water samples from P4 and P5 showed acute toxicity to *D. laevis* and *D. similis*, where *D. laevis* was more sensitive. These samples also caused mortality in *C. silvestrii* and *C. dubia*, within 48 hours in most assays, with higher effects in the dry season. Among the sampling sites, only P3 did not cause chronic toxicity to *C. silvestrii* and *C. dubia*, and these showed no significant differences in reproduction.

3.3 - Whole sediment toxicity tests

3.3.1 - Acute toxicity tests

There was no acute toxicity effect to *C. xanthus* at any site in any campaign. All organisms were alive at the end of the experiments, both in laboratory control and in samples tested.

3.3.2 - Chronic toxicity tests

In general, only samples from P4 and P5 caused mortality effect for *C. dubia* and *C. silvestrii*, within 48 hours in most assays, with no significant differences between species (Figure 5). Mortality was higher in the dry period.

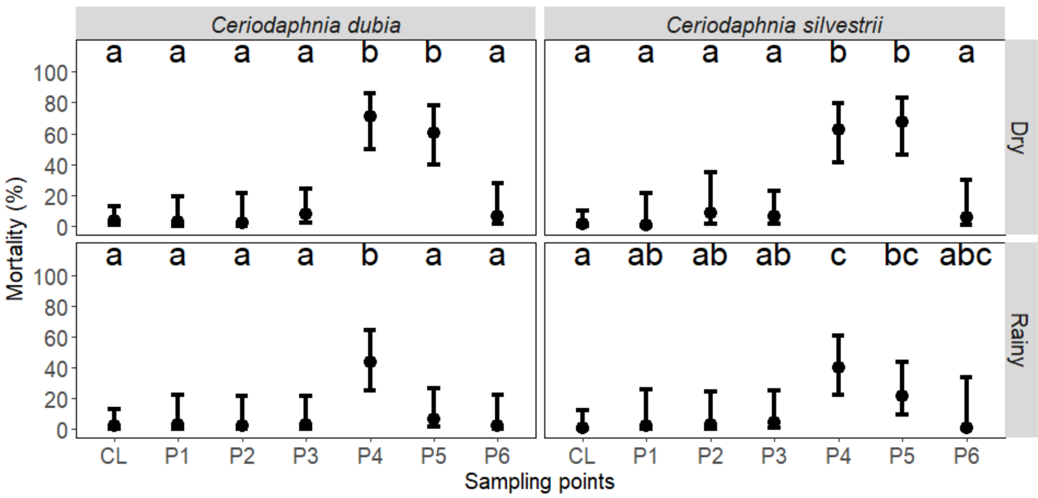
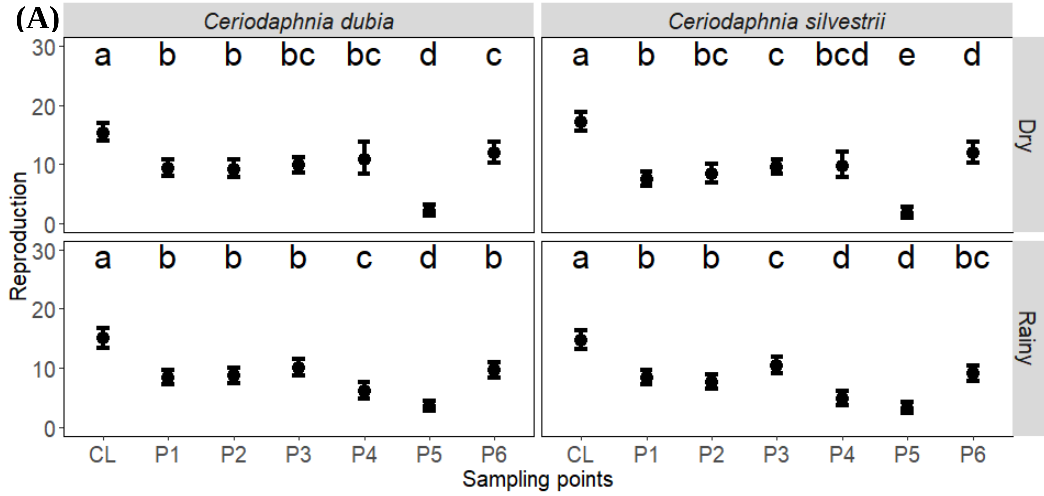


Figure 5 – Mean mortality rate in chronic toxicity tests with whole sediments from the different sampling sites conducted in the dry and rainy seasons. CL: laboratory control. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

There was no mortality in P4 and P5 in only one campaign in the dry season and in two in the rainy, which allowed the evaluation of the reproduction data of these sites.

All sites showed effect on *C. dubia* and *C. silvestrii* (Figure 6A), where P5 was the site of highest toxicity for both species. *C. dubia* and *C. silvestrii* reproduction was similar for each site (Fig. 6B), except in the dry season at P1, where *C. silvestrii* was more sensitive.

(A)



(B)

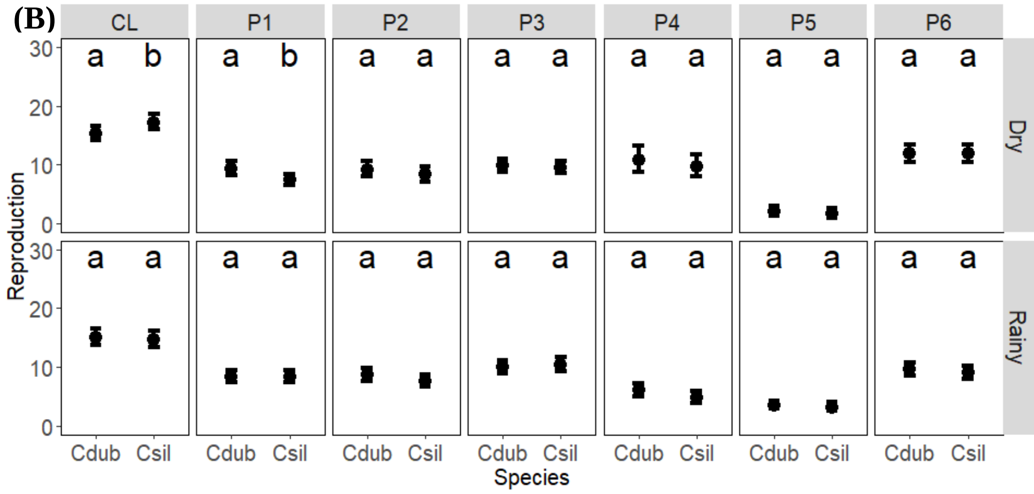


Figure 6 – Mean number of neonates produced in chronic toxicity tests with whole sediments from the different sampling sites, conducted in the dry and rainy seasons (A) and comparison between the species used in the two periods (B). CL: laboratory control; Cdub: *Ceriodaphnia dubia*; Csil: *Ceriodaphnia silvestrii*. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

In summary, sediment samples did not show acute toxicity effects to *C. xanthus*. On the other hand, samples from P4 and P5 caused mortality to *C. dubia* and *C. silvestrii*, within 48 hours in most assays, with higher effects in the dry season. All sites caused effects on reproduction for both species, including the site considered as field control. In general, there was no significant difference in reproduction between *C. dubia* and *C. silvestrii*.

3.4 - Comparison of chronic toxicity tests with water and total sediment

Figure 7 shows the comparison between the effects on reproduction in chronic toxicity tests with water and sediment. There were significant differences in reproduction between the two compartments, with higher toxicity caused by sediments at all sampling sites.

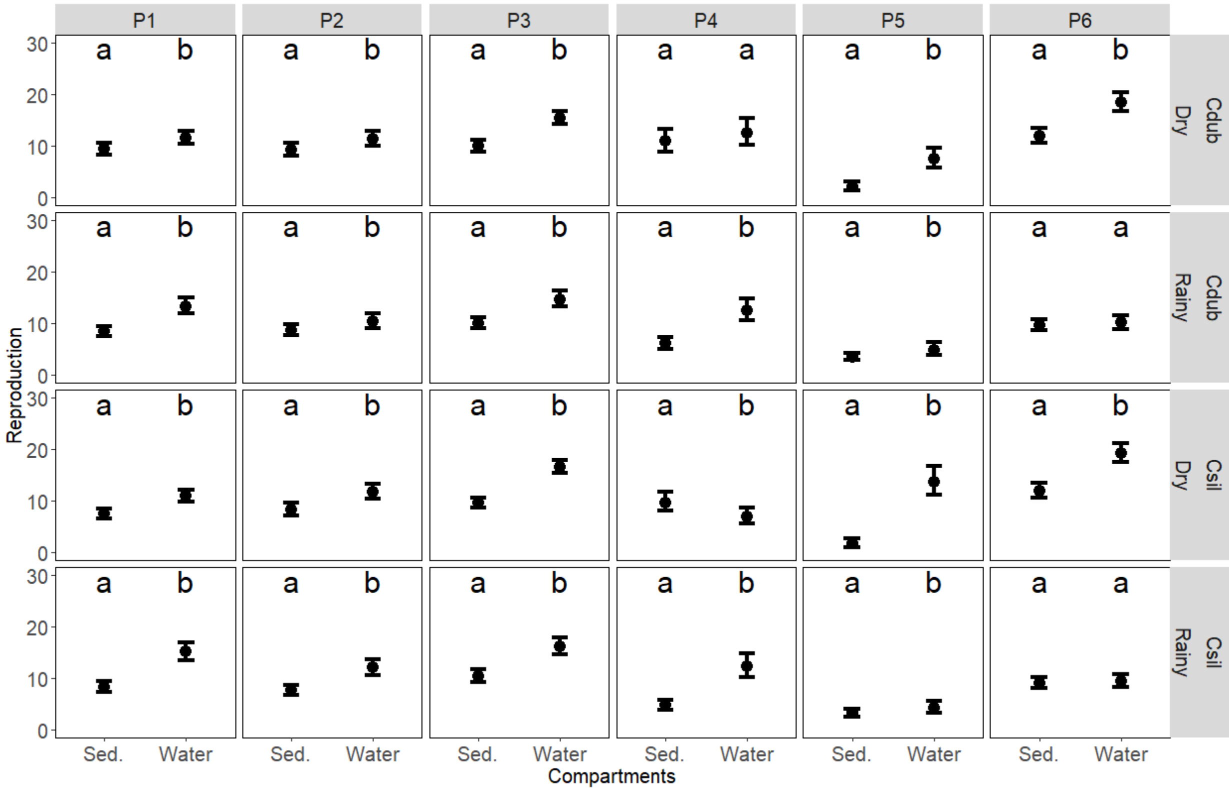


Figure 7 - Comparison of the mean number of neonates produced by *C. dubia* and *C. silvestrii* in the chronic toxicity tests with surface water and sediments from the different sampling sites, conducted in the dry and rainy seasons. Sed.: Sediment; Cdub: *Ceriodaphnia dubia*; Csil: *Ceriodaphnia silvestrii*. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

3.4 - Sediment elutriate toxicity tests

3.4.1 - Acute toxicity tests

Sediment elutriates from P4 and P5 caused acute toxicity to *D. laevis* in the dry season and only from P2 in the rainy season (Figure 8A). For *D. similis*, P1, P2, P3, P4 and P5 caused acute toxicity only in the dry season. *D. laevis* was more sensitive than *D. similis* in the case of P1, P2, P3, P5 and P6 in the rainy season and less sensitive in P1 and P3 in the dry season (Fig. 8B). Elutriates from P4 and P5 caused higher effects in the dry season.

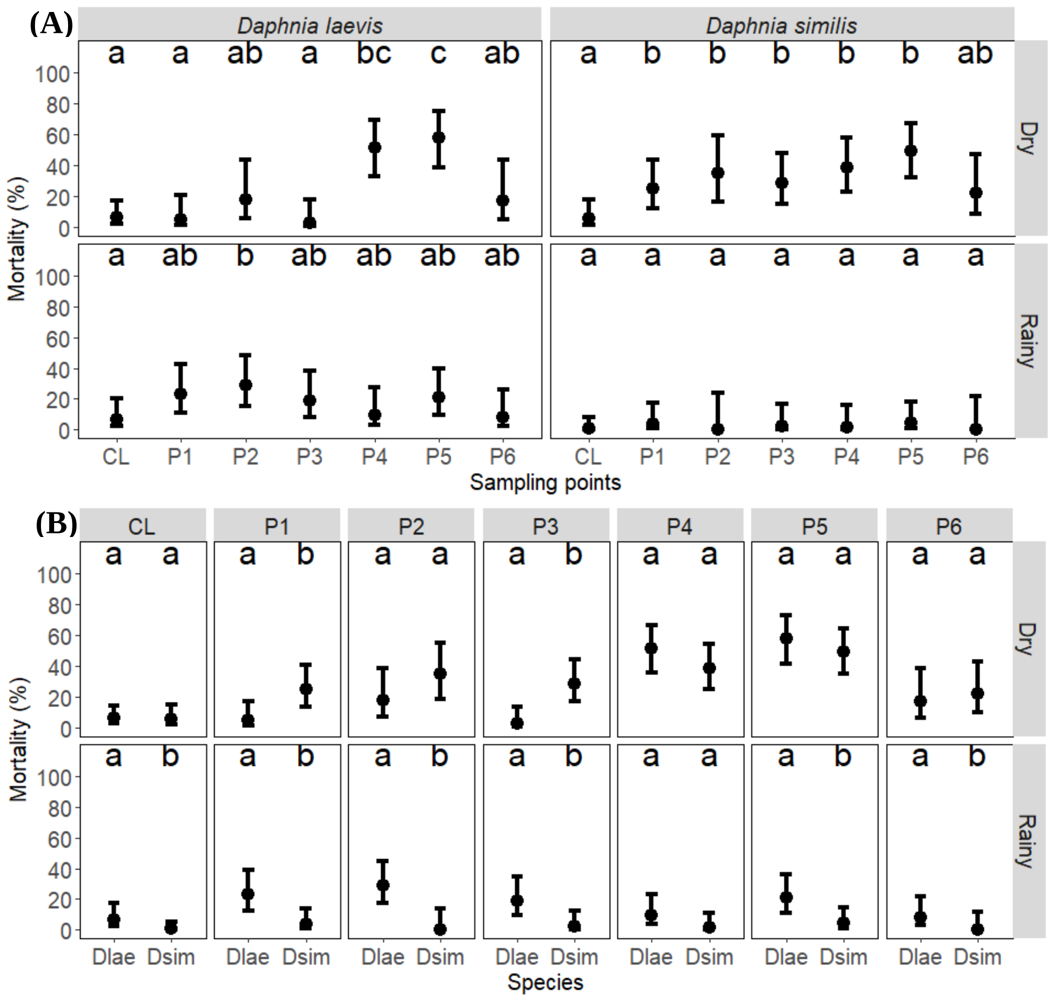


Figure 8 – Mean mortality rate in acute toxicity tests with sediments elutriates from the different sampling sites, conducted in the dry and rainy seasons (A) and comparison between the species used in the two periods (B). CL: laboratory control; Dlae: *Daphnia laevis*; Dsim: *Daphnia similis*. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

3.4.2 - Chronic toxicity tests

Elutriate samples from P4 and P5 caused mortality to *C. dubia* and *C. silvestrii* within 48 hours in two campaigns in the dry season, while P4 caused mortality to *C. dubia* in one campaign in the rainy period (Figure 9). The effects were higher in the dry season.

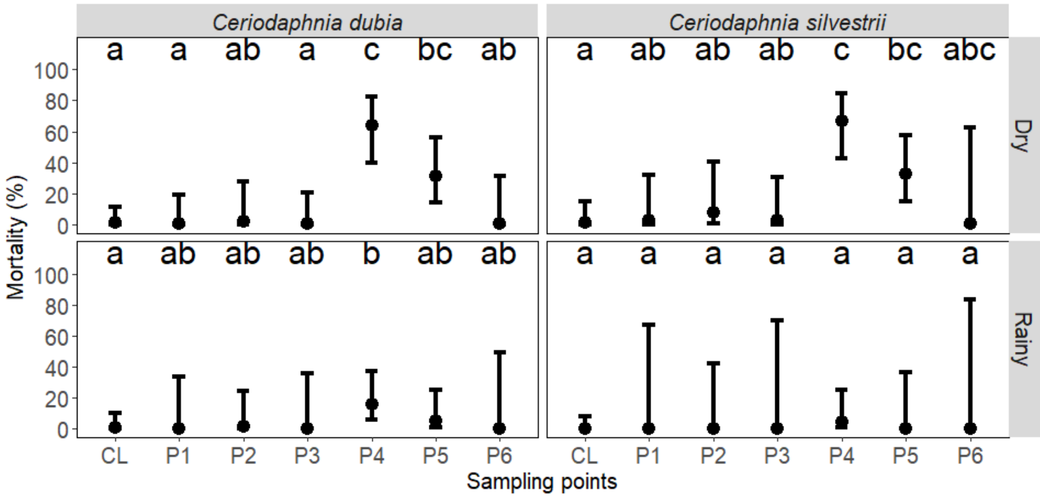


Figure 9 – Mean mortality rate in chronic toxicity tests with sediments elutriates from the different sampling sites conducted in the dry and rainy seasons. CL: laboratory control. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

Elutriate samples from P1, P2 and P5 showed reduced reproduction of *C. dubia* in both seasons and from P3, only in the rainy period (Figure 10A). For *C. silvestrii*, only P2 and P5 showed such effect in the dry period, while all sites caused toxicity in the rainy period. In general, *C. dubia* and *C. silvestrii* presented similar reproduction (Fig. 10B), except at P3 and P5 in the dry season, where *C. silvestrii* was more sensitive, and at P1 and P3 in the rainy period, where *C. dubia* was more sensitive.

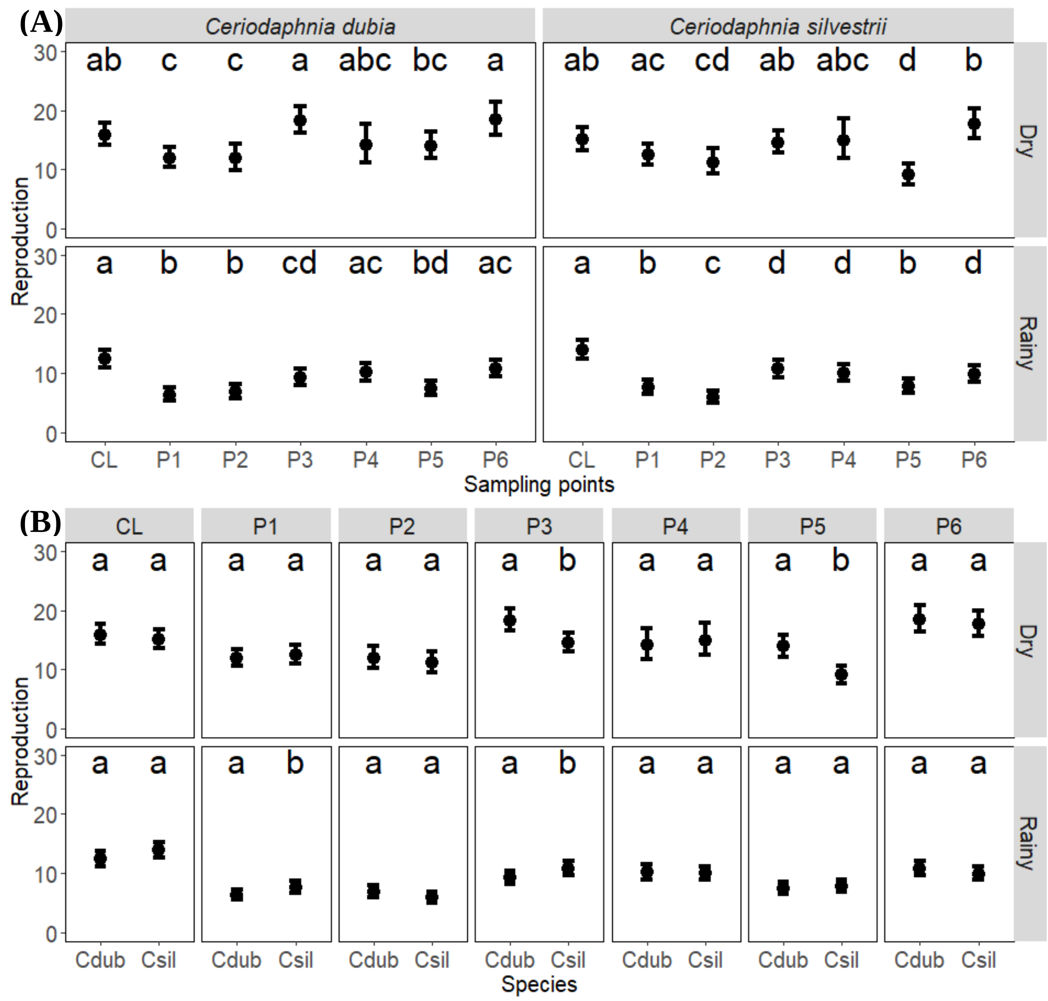


Figure 10 – Mean number of neonates produced in chronic toxicity tests with sediments elutriates from the different sampling sites, conducted in the dry and rainy seasons (A) and comparison between the species used in the two periods (B). CL: laboratory control; Cdub: *Ceriodaphnia dubia*; Csil: *Ceriodaphnia silvestrii*. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

In summary, sediment elutriates of P4 and P5 caused acute toxicity to *D. laevis* and *D. similis* in the dry season, and it had significantly different responses between the sites, without a defined pattern. P1, P2 and P5 caused higher chronic toxicity to *C. dubia* and *C. silvestri*i. P4 and P5 generated mortality in both species in the dry season. In general, *C. dubia* and *C. silvestrii* presented significant differences in reproduction between them only at P1, P3 and P5, without a defined pattern.

3.5 - Soil elutriate toxicity tests

3.5.1 - Acute toxicity tests

Soil elutriates of P3 and P4 caused acute toxicity effect to *D. laevis* in the rainy and dry seasons, respectively (Figure 11A). For *D. similis*, only P4 caused acute toxicity in the dry season. *D. laevis* was more sensitive than *D. similis* at all sites in the rainy period (Fig. 11B).

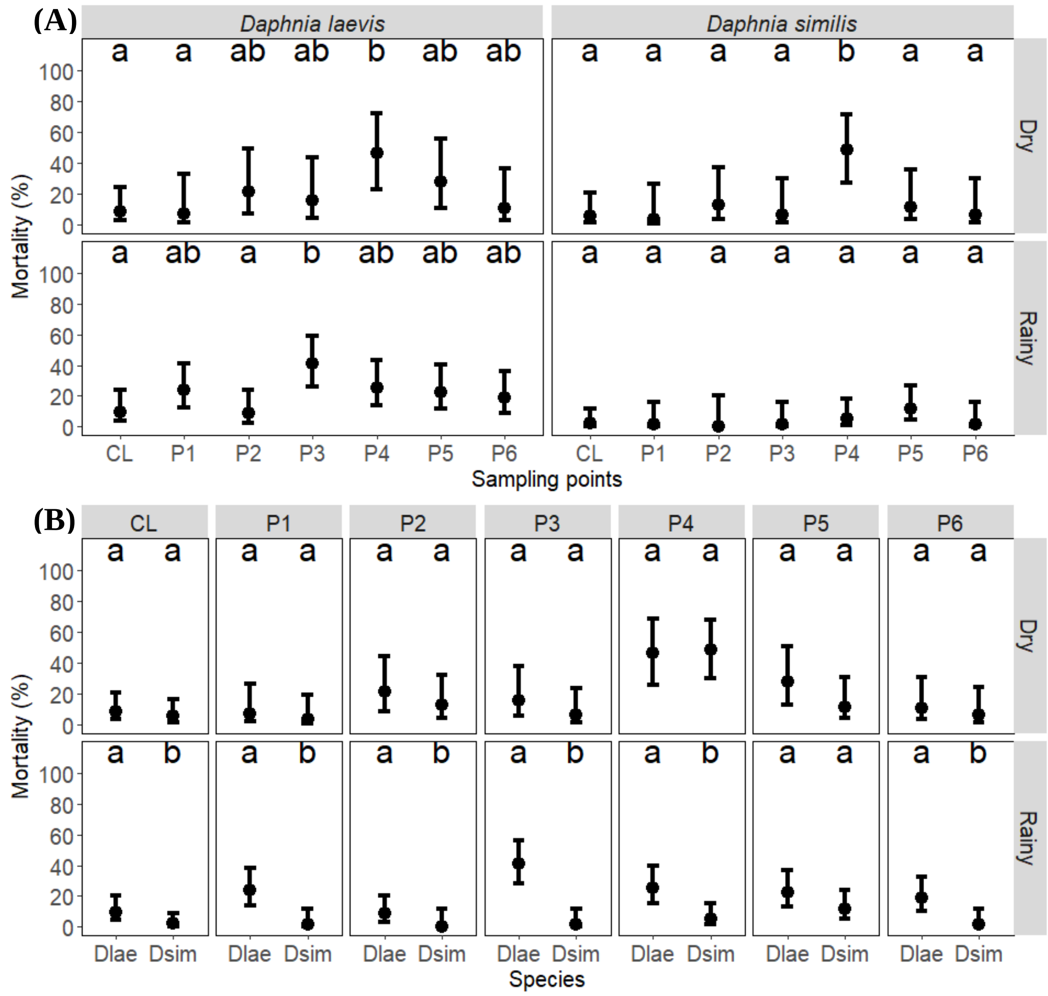


Figure 11 – Mean mortality rate in acute toxicity tests with soils elutriates from the different sampling sites, conducted in the dry and rainy seasons (A) and comparison between the species used in the two periods (B). CL: laboratory control; Dlae: *Daphnia laevis*; Dsim: *Daphnia similis*. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

3.5.2 Chronic toxicity tests

Elutriates of P1 and P4 caused chronic toxicity with effect on the survival for *C. dubia* in the dry and rainy seasons, and P3 only in the dry (Figure 12). For *C. silvestrii*, only P4 caused effect on the survival in the dry season and P1 in the rainy period.

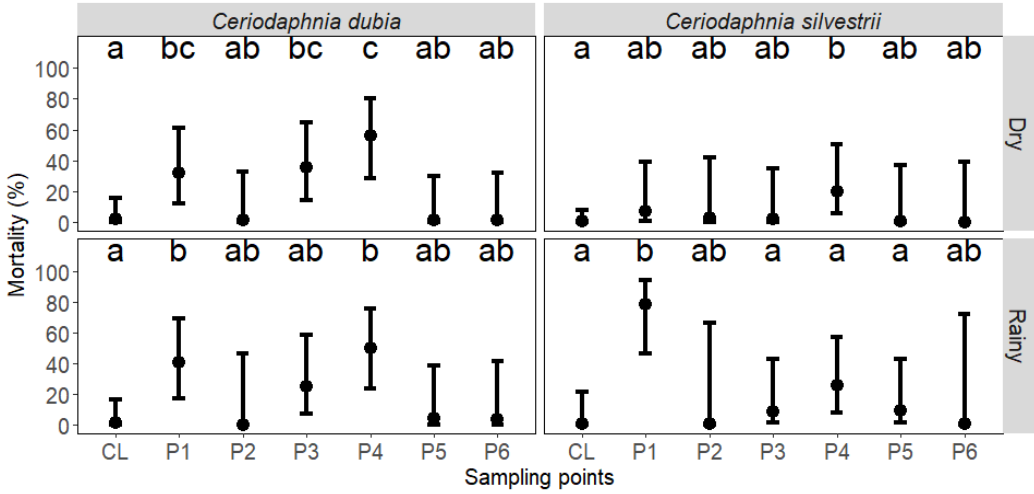


Figure 12 – Mean mortality rate in chronic toxicity tests with soils elutriates from the different sampling sites conducted in the dry and rainy seasons. CL: laboratory control. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

There was no mortality in all campaigns, which allowed the evaluation of the reproduction data of these sites.

Soil elutriates from P1 and P2 reduced the reproduction of *C. dubia* during the dry season and from P1 and P4 in the rainy period (Figure 13A). For *C. silvestrii*, P1, P3 and P5 caused this effect in both periods, while P4 and P6 only in the rainy season. Throughout the campaigns, the species showed significant differences in reproduction, although without a defined pattern among the sites and seasons (Fig. 13B).

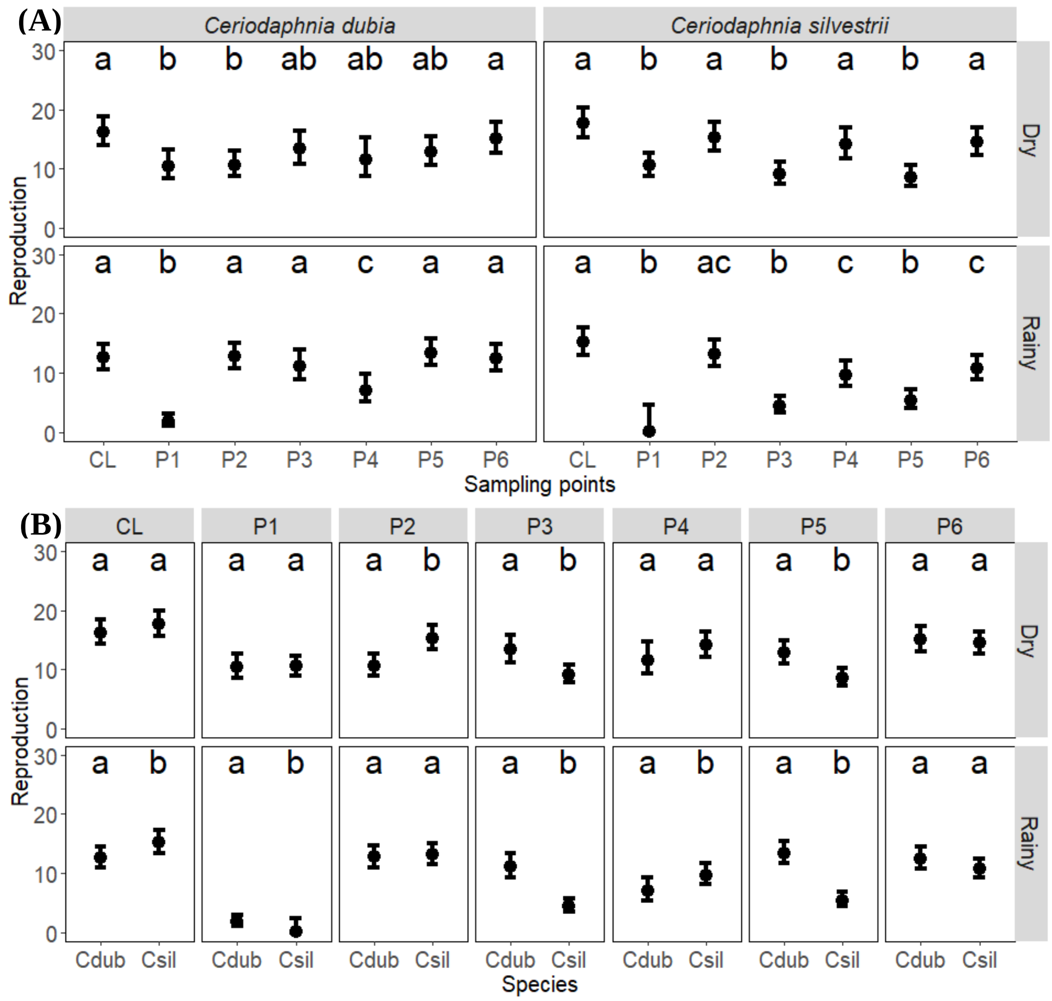


Figure 13 – Mean number of neonates produced in chronic toxicity tests with soils elutriates from the different sampling sites, conducted in the dry and rainy seasons (A) and comparison between the species used in the two periods (B). CL: laboratory control; Cdub: *Ceriodaphnia dubia*; Csil: *Ceriodaphnia silvestrii*. Treatments that share the same letter do not have significant differences between them, at 5% of significance.

In summary, soil elutriate of P4 caused acute toxicity to *D. laevis* and *D. similis* during the dry season, with *D. laevis* being more sensitive at all sites in the rainy period. P1 and P4 were the sites with higher chronic effects to *C. silvestrii* and *C. dubia*, which presented significant differences in reproduction, although without a defined pattern among the sites and seasons.

4 – Discussion

4.1 - Environmental monitoring in the ecotoxicological context

Considering the data obtained by IGAM at the same period of this study (IGAM, 2015a-c, 2016a-e, 2017a, b), it was verified that the site downstream of P1 showed good water quality, where only Mn concentrations was in disagreement with the standards of the Joint Regulatory Deliberation (RD) COPAM/CERH No. 01/2008 in some trimesters. In the present study, there were no metal concentrations above the limits of the RD in P1. However, acute and chronic toxicity effects of surface water were detected, characterizing the influence of iron mining activity on water quality at this site. According to Mendonça (2012), high concentrations of Fe and Mn in this area are due to the geological formation, which were potentialized in the last thirty years by anthropic intervention, such as the opening of roads, housing boom and iron mining. It should also be considered that the stream was impacted by the rupture of the tailings dam of the iron mining company, occurred on September 10, 2014.

IGAM data showed good water quality in P2, with high Fe concentrations and eventual fecal contamination. Our study also showed high Fe concentrations, but, in addition, the occurrence of chronic toxicity throughout the study period. IGAM data of P3, the field reference, showed anthropogenic influence due to the constant presence of *Escherichia coli*. Even with the concentrations of metals according to RD, acute toxicity to *D. similis* was verified in the dry period of 2016. On the other hand, according to IGAM (2016a; 2017a), the two sites presented excellent BMWP index in 2015 and 2016.

At site P6, IGAM data showed constant presence of *E. coli*, variation in contamination by toxic substances and predominantly absence of toxicity, differing from our study, in which, acute and chronic toxicity of water was verified. This site, in the metropolitan area of Belo Horizonte, is characteristic of high anthropic influence and disposal of contaminants in waterways.

Although, in the present study, P1, P2, P3 and P6 have shown metals and arsenic concentrations in water below the RD standards, acute and/or chronic toxicity effects were observed. The mean concentrations of metals quantified at these sites (Table 4) were below the median effective concentrations (EC50-48h) for *D. similis* for the elements As (450-540 $\mu g.L^{-1}$ - SALES *et al.*, 2016), Pb (527.5 $\mu g.L^{-1}$), Cu (1.9-289 $\mu g.L^{-1}$), Ni (832.2 $\mu g.L^{-1}$) and Zn (269.6 $\mu g.L^{-1}$) (MAGALHÃES *et al.*, 2014). The observed concentrations were also below the lowest observed effect concentration (LOEC) for *C. dubia* for Pb (80 $\mu g.L^{-1}$), Cu (15 μg.L^{-1}), Ni (3.8 μg.L^{-1}), Zn (128 μg.L^{-1}), Al (528 μg.L^{-1}) and Fe (960 μg.L^{-1}) and the maximum acceptable concentration (MATC) for As (1140 μg.L^{-1}) (USEPA, 2019). It is important to remember that water quality guides consider pollutants individually. However, the aquatic environment presents a complex mixture of chemicals whose toxicity may be underestimated (BALISTRIERI and MEBANE, 2014; WU *et al*., 2016). Metals can present toxicity in mixtures with concentrations below the legislation standards (COOPER *et al*., 2009). Different models have been developed to evaluate these effects, which due to their complexity have not been fully elucidated (NYS *et al*., 2016). Thus, the toxicity observed at these sites may be due to the metals mixture effects, or other contaminants not measured.

IGAM data of P4 showed high contamination by toxic substances, fecal contamination and toxic effects (acute or chronic) in all campaigns of the period considered. Hardness values above 1000 mg$CaCO_3$.L^{-1} and electrical conductivity above 2000 μS.cm^{-1} were quantified throughout the campaigns. Although increasing water hardness decreases the toxicity of metals, such as Ni, Zn, Cu and Cr, to cladocerans (CLIFFORD and McGEER, 2009; KOSLOVA *et al*., 2009; PARK *et al*., 2009), high hardness, electrical conductivity and sulfates can cause toxicity to these organisms (BOGART *et al*., 2016). Therefore, the water physical and chemical characteristics of P4 and P5, showed a set of factors responsible for the toxicity observed.

In the present study, water samples from P4 and P5 caused acute toxicity to *D. laevis* and *D. similis* and mortality of *C. silvestrii* and *C. dubia* within 48 hours in most assays, characterizing acute toxicity effect, with higher effects in the dry season. These sites have shown As, Cu, Ni, Zn, Fe and Mn concentrations above the RD standards (Table 4). Among these elements, the concentrations of As and Ni were below the $EC50_{48h}$ for *D. similis* (As: 450-540 μg.L^{-1}, Ni: 832 μg.L^{-1}), compared to the literature (MAGALHÃES *et al*., 2014; SALES *et al*., 2016). Likewise, the As concentrations were below the $EC50_{48h}$ for *C. silvestrii* (440-690 μg.L^{-1}) and *C. dubia* (1580-1720 μg.L^{-1}) (RAHMAN *et al*., 2014; SALES *et al*., 2016). However, in the case of Cu and Zn, the concentrations were above or around the $EC50_{48h}$ for *D. similis* (Cu: 13-43 μg.L^{-1}, Zn: 270 μg.L^{-1}) and *C. dubia* (Cu: 1-267 μg.L^{-1}, Zn: 60-1200 μg.L^{-1}) (USEPA, 2019), as well as for *C. silvestrii* (Cu: 5.65 μg.L^{-1} SANTOS *et al*., 2008). In addition, concentrations of Ni were above the $EC50_{48h}$ for *C. dubia* (4.8 μg.L^{-1} MAGALHÃES *et al*., 2014), showing that these metals may be related to the toxic effects observed on the cladocerans used in the present study.

The sites P4 and P5 are located, respectively, 900 and 200 meters downstream from the tailings pond of the gold processing plant. The results corroborated the negative ecological effects of gold processing activity, historically observed at this site by the water physical and chemical analysis (NONATO *et al*., 2007) and ecotoxicological studies of water, sediment and soil (SALES, 2013; ALVES and RIETZLER, 2015a).

Acute toxicity tests with sediment samples showed no mortality effect on *C. xanthus* larvae at any site evaluated. Chironomids are considered resistant to metal contamination (PINDER, 1986) and have been found in impacted sites of the study area (FEIO *et al*., 2015). However, studies have shown sub lethal effects, such as decreased larval growth and delayed adult emergence, in chironomids exposed to sediment samples contaminated by metals in gold mining areas (FARIA *et al*., 2007; CHIBUNDA *et al.* 2008). Moreover, morphological deformities (ALVES and RIETZLER, 2015b) and chromosomal abnormalities (SZAREK-GWIAZDA *et al*., 2013) related to metal contamination may also be observed in local populations. Thus, the acute toxicity tests may not be the most appropriate to assess the toxicity of these sediments on *C. xanthus*, being necessary to perform chronic toxicity tests for a better evaluation.

In chronic toxicity tests with whole sediment, all sites showed toxic effects to *C. silvestrii* and *C. dubia* throughout the monitoring period, even in P3, field reference. Samples from P4 and P5 caused mortality to both species, with higher effects in the dry period. Sediment toxicity was significantly higher than of the surface water. Thus, both toxicity assessments (surface water and sediments) showed that the study sites are in disagreement with RD COPAM/CERH No. 01/08, which establishes "non-verification of acute and chronic toxic effects to organisms in samples of water and/or sediment for rivers class 1 and 2”.

In an ecotoxicological study carried out in two areas of the Iron Quadrangle, Sales (2013) evaluated an area belonging to Doce river basin, which included three streams under influence of gold extraction activity. While for water, the sites presented different effects on cladocerans (acute, chronic and no effect), acute (*C. xanthus*) and chronic (*C. silvestrii*) toxicity of sediments were observed at all sites. In the second area, two streams were evaluated, one corresponding to P4 and other nearby, used for water supply. Chronic toxicity to *C. silvestrii* was observed in sediments from both sites and in water at P4.

Although surface water quality monitoring in Minas Gerais State does not contemplate sediments, several studies have shown the presence of metals and arsenic in high concentrations in this compartment in the Iron Quadrangle (BORBA *et al*., 2000; VIGLIO and CUNHA, 2010; VICQ *et al*., 2015). Ecotoxicological studies, however, are scarce.

Pereira *et al*. (2007) evaluated metals and arsenic concentrations in sediments at 20 sites in the Iron Quadrangle. Compared with the CONAMA Resolution No. 454/2012 standards, those concentrations were above level 2 at all sites for Cd; 18 sites for Ni (including areas close to all sites studied here); 16 sites for Cr (near P2, P3, P4, P5 and P6); 8 sites for As (near P4, P5 and P6); and intermediate values between levels 1 and 2 for Cu in 12 sites (near P2, P4, P5 and P6).

When performing the geochemical mapping of the Iron Quadrangle sediments, in order to determine reference values as a function of the region's lithology, Vicq *et al*. (2015) observed As, Cd, Cu, Ni, Cr and Zn anomalous concentrations due to the geological characteristic intensified by the mining activity. Comparing the data obtained here with the classification suggested by the author, there were anomalous concentrations of As at all sites and of Cd, Cu and Zn at P4 and P5.

Experiments with sediment elutriates showed that it is possible to predict the toxic effects of resuspension of the sediments evaluated. Elutriates of P4 and P5 caused acute toxicity to *D. laevis* and *D. similis* and mortality effects to *C. silvestrii* and *C. dubia*, in the dry season. There was a higher variability in reproduction with this phase than with whole sediment. While with whole sediment all sites caused chronic toxicity, elutriates of P3, P4 and P6 were not toxic in some campaigns, besides smaller effects.

One reason for this difference could be the elutriate preparation. Elutriate tests are more likely to present a false negative result, since exposure is limited mainly to pollutants that easily dissociate from sediment particles, where the contact time between water and sediment can exert substantial effects, thereby limiting the overall exposure (BURTON, 1991; HARING *et al*., 2012). However, studies have successfully used elutriate to verify toxic effects of sediments to aquatic species in deactivated mining areas (VIDAL *et al*., 2012) or remediation projects (NOLLER *et al*., 2013), and to identify the compounds responsible for toxicity in metal-contaminated reservoirs (RIETZLER *et al*., 2016).

The predominant absence of water toxicity in P3; variability of effects at other sites in some campaigns (P1, P6); constant sediment chronic toxicity at all sites; and sediment elutriates toxicity, together with the data obtained by Sales (2013), corroborate the sediments conservative and source of contaminants characteristics, demonstrating the need of sediment monitoring in mining areas. Monitoring the sediment quality may indicate changes in the contaminants accumulation rate, benthic community structure and toxicity, providing important information for management and aquatic ecosystems recovery (ARAÚJO *et al*., 2006). Thus, monitoring sediment quality can provide useful data for environmental forensic investigation.

With respect to soil samples, the As concentrations at all sites and Cr and Ni in P3, P4, P5 and P6, suggest changes in soil quality with potential to impair their functions. This was confirmed by soil elutriate toxicity tests, where all sites presented at least one chronic toxicity event. These soils have low retention capacity, with potential risk of pollutants leaching to ground and surface waters. The differences in results between the campaigns, related to toxicity and metals concentrations, may be due to the sampling site, since the sorption potential can vary considerably even in a small area, due to soil heterogeneity, resulting in differences in contaminants availability (NIEMEYER *et al*., 2010).

The As solubility in soils is influenced by iron and aluminum oxides, to which As has strong adsorption, particularly to iron oxides. Changes in environmental conditions, such as rainy periods or flood events, may cause metal oxides dissolution and consequently mobilization of sorbed contaminants, such as As (ROCHA *et al*., 2011). This condition could have been induced by the elutriate preparation method in the present study, being important to consider the toxic effects of metals mixture, where, for example, As and Fe may have synergistic effects on *D. similis* and *C. silvestrii* (SALES *et al*., 2016). However, the toxicity differences among the sampling sites may be related to the characteristics of each soil (composition of the clay fraction, chloride ions, organic matter and manganese oxides) (LOUREIRO *et al*., 2005).

Soil elutriates of P4 showed the highest toxicity effects, with mortality in both acute and chronic toxicity tests and decrease in reproduction. In the ecotoxicological assessment of soils around two mining areas, one corresponding to P4 and another in the Doce river basin, Alves and Rietzler (2015a) observed toxic effect on reproduction in *Eisenia andrei* in both areas, correlated with high As concentrations, implying in degradation of the habitat function of these soils. In addition to effects on the biota, high As concentrations have already been detected in children living in this region, as intake of soil dust a likely source of contamination (MATSCHULLAT *et al*., 2000). It emphasizes the need of soil stabilization against wind and water erosion (DESCHAMPS *et al*., 2002), in order to reduce its potential for contamination, characterized by low retention capacity observed in this study.

The environmental impacts of mining activities have also been the subject of research internationally. Although many studies have considered only geochemical evaluations of surface waters, sediments and soils, showing high concentrations of metals (ESPAÑA *et al*., 2005; ALEKSANDER-KWATERCZAK and HELIOS-RYBICKA, 2009; HUANG *et al*., 2010; NING *et al*., 2011; LOPES *et al*., 2015), studies of local communities showed lower species diversity, teratological effects and bioaccumulation due to metals exposure (HIRST *et* *al*., 2002; SILVA *et al,* 2009; NIEMEYER *et al*., 2012; ALLERT *et al*., 2013). In addition, the ecotoxicological approach has been used to assess water and sediment quality of rivers under mining activities influence (ANTUNES *et al*., 2007; LATTUADA *et al*., 2009; GERAS'KIN *et al*., 2011). With respect to sediments, studies have used whole sediment, pore water (BESSER *et al*., 2009) and elutriate (VIDAL *et al*., 2012), with bioindicators of different trophic levels.

In this context, it is clear the need of more sites concerned with ecotoxicological assessment of water, as well as the importance of implementing sediment and soil toxicity assessment in environmental monitoring, especially in mining areas. Thus, more complete information on the quality of regional ecosystems can be obtained, a useful tool for environmental forensic investigation.

4.2 - Test organisms in environmental monitoring

Bioindicators used in the environmental assessment of areas under influence of mining activities have identified from absence of toxicity to lethal and/or sublethal toxic effects. In a uranium mining area, Antunes *et al*. (2007) observed water toxicity at different intensities, considering *Daphnia longispina*, *Pseudokirchneriella subcapitata* and *Daphnia magna*, and absence of whole sediment toxicity to *Chironomus riparius* as well as elutriate to daphnids. Lead mining impacts on survival and reproduction were observed in whole sediment tests with *Hyalella azteca* and *C. dubia* in pore water (BESSER *et al*., 2009). Lattuada *et al*. (2009) identified water and sediment acute toxicity to *D. magna*, while Geras'kin *et al*. (2011) verified genotoxic effects to *Allium cepa* in water and sediment in coal mining areas.

Niemeyer *et al*. (2010) and Niemeyer *et al*. (2015) conducted an ecological risk assessment in an abandoned mining area considering avoidance and reproduction tests with terrestrial organisms, plant growth and biomass, and acute and chronic toxicity tests with aquatic species, including *D. magna*. The authors verified absence of toxicity to aquatic organisms, suggesting a high soil retention capacity. Nonetheless, experiments with other organisms showed toxicity effects, resulting in high local risk. Some other studies have shown that *D. magna* presented acute and/or chronic toxicity effects to soil elutriates from mining areas, while other terrestrial species (LOUREIRO *et al.,* 2005; ALVARENGA *et al*., 2008) and plants (SANTOS *et al*., 2013) did not indicate toxic effects. In this sense, the latter authors emphasized the sensitivity and importance of bioassays with soil elutriates, reinforcing the need to perform bioassays with different organisms in environmental monitoring.

Acute toxicity tests with *D. laevis* and *D. similis* for surface water and elutriates of sediments and soils showed variability of responses between species in relation to sampling sites and seasonality. In water and whole sediment chronic toxicity tests, there were no differences in sensitivity between *C. dubia* and *C. silvestrii*. In relation to sediment and soil elutriates, the species showed variations in sensitivity among sampling sites and campaigns. However, higher sensitivity was observed for *D. laevis*, native species, especially in the most contaminated sites. There were also differences in responses between *Daphnia* spp. and *Ceriodaphnia* spp., where water and sediment elutriates caused acute toxicity, but did not cause chronic toxicity in some campaigns. Therefore the adoption of more than one type of ecotoxicological testing, with different organisms, may provide greater consistency and comprehensiveness to the environmental monitoring of Minas Gerais, comparable, for example, to the monitoring conducted in the state of São Paulo (CETESB, 2017).

The protocols for ecotoxicological assessments and the water, sediment and soil quality standards used in tropical countries are derived from the temperate region countries (BERTOLETTI and ZAGATTO, 2006; NIEMEYER *et al.*, 2015). However, the species sensitivity of the two regions may be different for some substances (KWOK *et al*., 2007; DAAM and VAN DEN BRINK, 2010). *C. silvestrii* and *C. dubia* presented similar sensitivities to sodium chloride and sodium dodecyl sulfate (RIETZLER *et al*., 2017). However, *C. silvestrii* was more sensitive to pesticides than *C. dubia* (MANSANO *et al*., 2016). Rietzler *et al*. (2017) observed higher sensitivity of *D. laevis* to potassium dichromate and sodium chloride compared to *D. similis*, and similar sensitivity to sodium dodecyl sulfate. In relation to copper sulfate, *D. laevis* was less sensitive than *D. similis* and *D. magna* (ARAUCO *et al*., 2005). Regarding metals, the sensitivity difference among species is an important ecotoxicological aspect of cladocerans (WANG, 2013).

In this context, studies have been carried out aiming to obtain data related to cultivation, life cycle and sensitivity to various substances of cladocerans from tropical regions as *D. laevis*, *C. silvestrii* (FONSECA and ROCHA, 2004b; JACONETTI, 2005; RIETZLER *et al*., 2017; MANSANO *et al*. 2016), *Ceriodaphnia cornuta* (RIBEIRO, 2011; RIETZLER *et al*., 2017), *Pseudosida ramosa* (FREITAS and ROCHA, 2011), *Moinodaphnia macleayi*, *Ceriodaphnia rigaudii* and *Diaphanosoma brachyurum* (MOHAMMED and AGARD, 2006). The same approach has been taken in relation to benthic organisms, as *C. xanthus* (FONSECA and ROCHA, 2004a; SANTOS *et al*, 2007; NOVELLI *et al*., 2012;

CAMPAGNA *et al*., 2013., RIETZLER *et al*., 2017), *Branchiura sowerbyi* (ALMEIDA, 2007; DUCROT *et al*., 2010; DHARA *et al*., 2015), *Hyalella meinerti, H. curvispina*, *H. pleoacuta* e (ARAÚJO, 1998; DUTRA, 2007).

D. laevis and *C. silvestrii* have often been used in ecotoxicological assessments applied to effluents and surface waters (FONSECA, 1991; ALVES and RIETZLER, 2014; RIETZLER *et al*., 2016). *C. xanthus* has been used to assess sediment quality of rivers and reservoirs (SILVÉRIO *et al*., 2005; DORNFELD *et al*., 2006), and other studies have also conducted tests with *C. silvestrii* in addition to *C. xanthus* (JANKE *et al*., 2011; YAMADA *et al*., 2012; ALVES and RIETZLER, 2015b; SUEITT *et al*., 2015). Sublethal effects have also been reported in *C. xanthus*, such as morphological deformities, genotoxicity and bioaccumulation in individuals exposed to cyanotoxins (EMYGDIO, 2011; SANTIAGO, 2012), as well as changes in growth and emergence due to pesticides (PRINTES *et al*., 2011; FERREIRA JUNIOR *et al*., 2017).

Studies conducted in mining areas in Brazil and other tropical countries used exotic species in ecotoxicological assessment of water, sediment (CHIBUNDA *et al*., 2008; LATTUADA *et al*., 2009; NOLLER *et al*., 2013) and soils (NIEMEYER *et al*., 2010, 2015; ALVES and RIETZLER, 2015a). However, the use of local species as bioindicators was highlighted in studies assessing the toxicity of wastewater (VAN DAM *et al*., 2008) and sediments (MEHLER *et al*., 2019), in gold mining areas in Australia.

The recurrence of toxicity to *D. laevis* and *D. similis* at sites P4 and P5 showed that the use of acute toxicity tests could be sufficient to evaluate sites highly contaminated by mining activities. Also, *D. laevis*, proved to be an efficient tool for environmental forensic investigation in these areas.

Considering that *C. silvestrii* showed to be as sensitive as *C. dubia* in surface water experiments and sensitivity differences with elutriates, it is emphasized the importance of using native species in ecotoxicological assessments in order to obtain a more representative assessment of pollutants effects on local biota. In addition, one should avoid the risk of accidental introduction of exotic species from temperate regions into tropical ecosystems, where they can become invasive and dangerous to local biodiversity (FREITAS and ROCHA, 2011; MANSANO *et al.*, 2016).

5 – Conclusions

Water toxicity tests showed lethal and/or sublethal effects at sites where metals concentrations were in accordance with the legislation, demonstrating the importance of ecotoxicological assessment coupled with chemical analyses in environmental monitoring. They also showed the need of more sites concerned with the ecotoxicological assessment, particularly in mining areas. It was evidenced by P4 and P5, especially in the dry season.

The adoption of more than one type of ecotoxicological testing, with different organisms, as well as the use native species should be considered in addition to the chronic toxicity tests already carried out on the monitoring of Minas Gerais watersheds. They may provide a more realistic assessment of the pollution effects to the biota.

Whole sediments must certainly be included in ecotoxicological monitoring and evaluation of water bodies. Elutriate tests have also showed effects caused by sediment resuspension, and can also be included.

The soils from the Iron Quadrangle showed low retention capacity. They reinforce the need of integrating ecotoxicological studies to soil geomorphology, vegetation cover and geochemical properties, which influence the natural mobility of the potentially toxic elements in those sites.

The recurrence of acute/chronic toxicity of water and sediments to cladocerans at sites with direct influence of mining activities has shown that the ecotoxicological approach provides consistent results for use as evidence for environmental forensic investigation in mining areas.

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6 - CAPÍTULO 2: Chironomids as indicators of contamination in gold processing areas: sublethal effects of sediments on *Chironomus xanthus*.

Abstract

Mining activity is a major source of pollution by metals and metalloids that accumulate in the sediment at high concentrations potentially toxic to biota. Sediment toxicity tests using benthic macroinvertebrates may reveal the adverse effects to organisms caused by sediment contaminants. In this context, toxic effects of sediment from surroundings of a gold processing area on *Chironomus xanthus* were evaluated, emphasizing the partial life cycle (growth, emergency), avoidance behavior and bioaccumulation as endpoints. Bioaccumulation was also evaluated in organisms collected in the field. Avoidance experiments did not show to be a good tool to evaluate sediments contaminated by metals. On the other hand, growth (dry weight) and time of emergency of *C. xanthus* larvae exposed to sediment samples were significantly delayed compared to controls in all experiments. The ability of *C. xanthus* to bioaccumulate metals, as well as that of indigenous organisms, has also been shown. Thus, most sublethal toxic effects demonstrated the impacts of mining activity on a benthic organism, providing tools for environmental monitoring and forensic investigation.

Keywords: Ecotoxicology; mining activity; sublethal endpoints; benthic macroinvertebrates; bioaccumulation; environmental monitoring.

1 – Introduction

Sediment is an important compartment for maintaining the quality of aquatic ecosystems, providing areas of habitat, feeding and spawning for many aquatic organisms. However, it is also a reservoir of pollutants, which may present toxic substances in concentrations much higher than in the water compartment. It is, therefore a source of contamination for aquatic life and humans by bioaccumulation in the food chain (SIMKISS *et al*., 2001; USEPA, 2000, 2001).

Mining activity is a major source of pollution by metals and metalloids, both in the extraction and processing of the ore. Its effects can last for centuries or even millennia after closure of activities, as well as reaching the biota at long distances downstream from the source of contamination (BESSER *et al.,* 2015; YOUNGER and WOLKERSDORFER, 2004). When their effluents containing metals reach water courses with a more neutral pH, metals tend to precipitate due to the decrease of solubility and strong affinity for solid phases (SALOMONS, 1995). Thus, metals with potential toxic effects accumulate in the sediment at concentrations hundreds of times larger than in the water column, with possible exposure to benthic organisms (SIMKISS *et al*., 2001).

Sediment toxicity tests using benthic macroinvertebrates may reveal the occurrence of adverse effects to organisms caused by contaminants in sediment (SIBLEY *et al*., 1997), providing scientific evidence to meet legal and regulatory proceedings, supporting management decisions (ALVES and RIETZLER, 2015; BESSER *et al*., 2015). Several parameters can be used to assess these effects. Among them, sublethal parameters, such as survival, growth, reproduction and behavior may be more sensitive than lethality to assess the effects of contaminants on populations and communities (BENOIT *et al*., 1997; KALINOWSKI and ZAŁĘSKA-RADZIWIŁŁ, 2011).

Contaminants can also accumulate in organisms and do not always manifest detectable acute or chronic effects. The effects can be recognized in a later phase of life, showing multi-generation effects or manifest only in higher levels of the food web. Therefore, bioaccumulation should be considered among the criteria for assessing adverse effects on the ecosystem (FRANKE *et al*., 1994).

Widely distributed, benthic macroinvertebrates of the Chironomidae group are one of the most abundant organisms in the benthic community, having great ecological importance (PINDER, 1986; PÉRY *et al*., 2002). They are considered good indicators in evaluations of sediment toxicity, because they live directly in contact with the sediment and feed on debris

(DICKMAN *et al*., 1992). Among them, *Chironomus tentans* and *Chironomus riparius* are extensively used, with protocols already defined for acute, chronic, life cycle and bioaccumulation toxicity tests (USEPA, 2000; ASTM, 2005; OECD, 2010, 2011).

Chironomus xanthus Rempel, 1939 (Diptera, Chironomidae) is an endemic tropical species widely used in ecotoxicological studies in Brazil (FONSECA and ROCHA, 2004; SILVÉRIO *et al*., 2005; CAMPAGNA *et al*., 2013; ALVES and RIETZLER, 2015; RIETZLER *et al*., 2017). Its laboratory culture is well established, presenting easy maintenance, high reproduction and survival rates and short life cycle of about 13 days, making them an adequate species as test-organism (FONSECA and ROCHA, 2004). *C. xanthus* presents a faster development than *C. tentans* and *C. riparius*, allowing experimental results in a shorter period.

The study area belongs to the Iron Quadrangle, Minas Gerais State - Brazil, downstream of a gold ore processing area. The studied stream has historical toxic effects of water (IGAM, 2016, 2017) and sediment (SALES, 2013) on cladocerans, and absence of acute toxicity on *C. xanthus* (SALES, 2013). Based on that, this study aimed to evaluate the toxicity effects of sediment of a gold processing area on *C. xanthus*, emphasizing the partial life cycle (growth, emergency), avoidance behavior and bioaccumulation as endpoints, as well as the use of these tools for future environmental monitoring and forensic investigation.

2 – Material and Methods

2.1 - Sediment sampling and characterization

Sediment samples were collected downstream of a gold ore processing area in the Iron Quadrangle, Minas Gerais State, Brazil (Figure 1). This area was chosen for the greatest toxicity effects in the experiments described in Chapter 1. At the site closest to the dam (P5: 19° 58' 29.1"S, 43° 49' 41.5"W), samples were collected in the dry (June and August/2017) and rainy (March and April/2018) seasons, to evaluate growth, emergency, avoidance behavior and bioaccumulation of metals with *Chironomus xanthus* as test organism. A second sampling site (P4: 19° 58' 45.1"S, 43° 49' 15.2"W) was included in the rainy season to evaluate bioaccumulation of metals in native benthic macroinvertebrates, since it was not found in samples of P5. Surface sediments were collected in polyethylene pots in a backwater area on the stream bank. Samples were transported to the laboratory in a thermal box with ice and kept in a refrigerator at 4°C, according to NBR 15469 (ABNT, 2015).

Granulometric analysis was performed using the Pipette Method, which consists of separating the silt, clay and sand by mechanical agitation of the sample with a chemical dispersant, followed by decanting and separating the clay fraction by pipette and the sand by sieve. After drying and weighing, the percentages of each fraction are determined. The percentage of organic matter was assessed by oxidation of organic matter with potassium dichromate on heating. The excess dichromate is titrated with standard solution of ammoniacal ferrous sulfate (EMBRAPA, 1997).

Determination of metals and metalloid bioavailable in sediments followed Method 3051A (USEPA, 2007) for digestion with nitric acid in microwave (MARS-CEM). Metal quantification was performed by inductively coupled plasma optical emission spectrometry (ICP-OES Spectro Spectroflame 4165/91). Quantification of arsenic was done by inductively coupled plasma mass spectrometry (ICP-MS Perkin Elmer ELAN DRC-e).

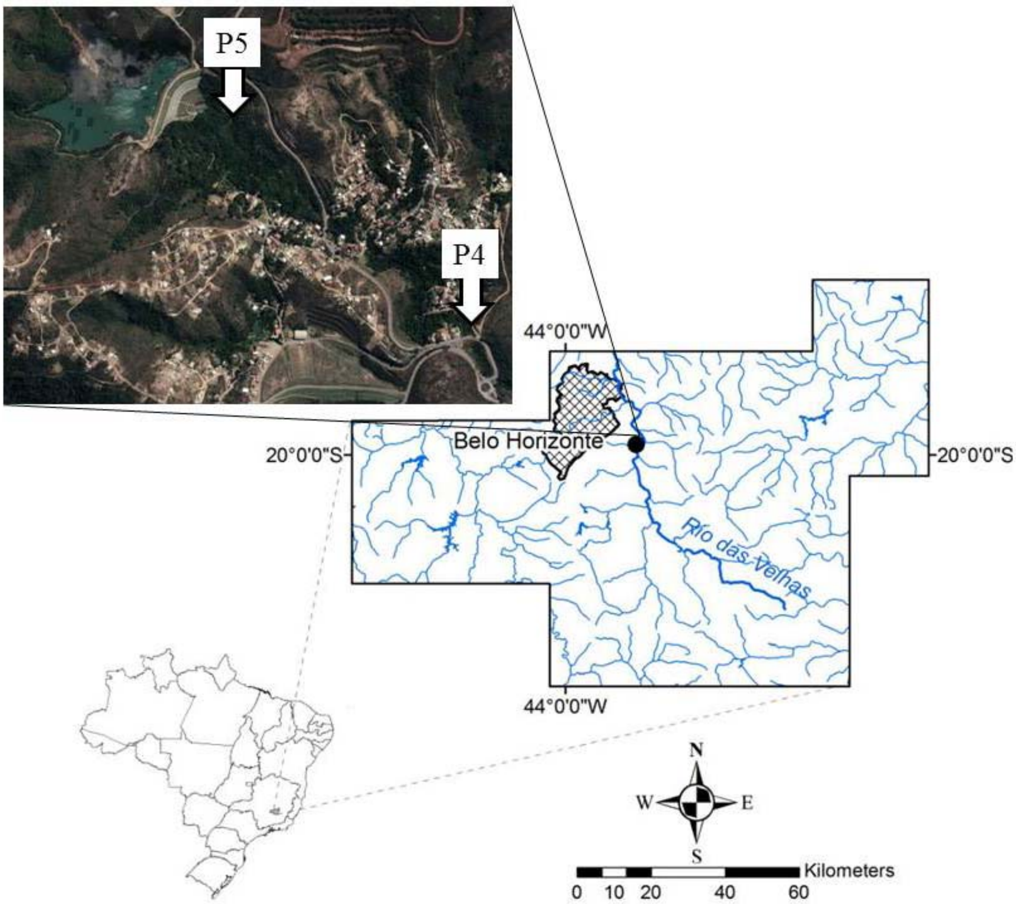

Figure 1 – Study area in Iron Quadrangle, Minas Gerais, Brazil, highlighting the city of Belo Horizonte.

2.2 - *Chironomus xanthus* culturing

C. xanthus were maintained in the Laboratory of Ecotoxicology, Institute of Biological Sciences, Federal University of Minas Gerais, based on Fonseca and Rocha (2004). Cultures were initiated by transferring approximately 100 first instar larvae to plastic trays (45x35x6cm) containing one part of calcinated sand and four of water. Larvae were fed daily with distilled water suspensions of fish food (0.04 $mg.ml^{-1}$) plus an algal suspension of *Raphidocelis subcapitata* (10^5 $cells.ml^{-1}$) only on the first day. The trays were covered by tulle screens (35cm height) to retain adults emerged, allowing copulation and deposition of the spawnings. Cultures were kept at 24 ± 1ºC and photoperiod of 12 hours, under constant aeration. Culture water, obtained from an uncontaminated natural spring, has a neutral pH, hardness between 32-36 $mgCaCO_3.L^{-1}$, dissolved oxygen around 6-7 $mg.L^{-1}$ and electrical conductivity of 160-180 $\mu S.cm^{-1}$.

2.3 - Partial life cycle experiments

Partial life cycle experiments were conducted with sediments from P5, based on USEPA (2000). Twelve replicates were prepared by placing 50 g of sediment and 200 ml of culture water (added slowly to minimize suspension of the sediment), one day before introduction of larvae. They were kept without aeration to enable the sedimentation and stabilization of the system. On the following day, slow aeration was started prior to the introduction of organisms and maintained throughout the experiment. Five first instar larvae were introduced in each pot.

Feeding was done as described for culturing the organisms. The experiment was kept at 24 ± 2ºC and photoperiod of 12 hours. Controls used the same sediment used for culturing at the laboratory, with granulometry similar to the environmental samples.

On the 8^{th} day, four replicates were taken for survival and growth (dry weight) measurements. The sediment was sieved and the surviving larvae were kept individualized in culture water for one night in order to empty the digestive tract. The organisms were then individually placed in previously weighed aluminum crucibles, taken to the oven at 60°C for 24 hours and then weighed (CHIBUNDA *et al*., 2008).

The eight remaining replicates were covered with screens for adults retention and checked daily for accounting of adults emerged. Only adults completely released from exuviae were considered emerged. The experiments were ended within a maximum of 15 days.

Two experiments were conducted at each campaign (Jun1, Jun2; Aug1, Aug2; Apr1, Apr2). In March, only one experiment was conducted (Mar). In Aug2 and Apr2 experiments, replicates of the environmental sample were included exposing the larvae from the reproduction (1^{st} generation) of the organisms exposed to the environmental sample in Aug1 and Apr1, respectively.

2.4 - Avoidance experiments

Rectangular plastic recipients (14.5x10x4.5cm) were used, where 50g of sediment from P5 was added in one half of the recipient and 50g of culture sediment in the other half. The sides were separated by a plastic divider and 200 ml of culture water was added on each side at the same time. A plastic was placed over the sample to minimize the suspension of material, being removed after water addition. After the sediment had settled, the divider was also removed.

On a line between the two sediments, slowly aeration was started on one side of the recipient and the organisms added on the other side. After 96 hours, water was carefully removed and the sediment sieved to count larvae found on each side.

In order to verify if the distribution of organisms would be homogeneous (negative control), recipients were set up only with the control sediment on both sides (dual control tests). The same was done with the environmental sample (dual sample).

Three replicates were used with ten second instar larvae in each, fed daily. One experiment per campaign was conducted (Jun, Aug, Mar, Apr). The experimental design was based on Wentsel *et al*. (1977) and De Hass *et al*. (2006).

2.5 - Bioaccumulation

Metals and metalloid bioaccumulation was evaluated only in the rainy season.

C. xanthus larvae from the laboratory were exposed to the P5 sediment samples. Two plastic trays were prepared with one part of sediment sample and four of culture water, one day before introduction of larvae. On the following day, slow aeration was started prior to the introduction of organisms and maintained throughout the experiment. Approximately 100 first instar larvae were transferred to each tray. Feeding was done as described for organisms culturing. The system was maintained at 24 ± 1ºC and photoperiod of 12 hours.

On the 8th day, the sediment was sieved (0.5mm). The surviving larvae were individualized in culture water to empty the digestive tract. The larvae were held for 10 minutes in a solution of EDTA 1g.L^{-1} to remove metals from the surface of the body (PÉRY *et al*., 2008), washed with distilled water and frozen. Samples were lyophilized and the dried tissue was digested in nitric acid and hydrogen peroxide, based on Method 200.3 (USEPA, 1991). Samples were filtered with syringe filter (45 µm) prior to metals quantification on ICP-MS.

Sediment samples from P4 were sieved (0.25mm) upon arrival in the laboratory. The organisms found followed the same process described above to cleaning and quantification of metals. In both campaigns only Chironomidae and Oligochaeta were found, which were pooled to obtain sufficient biomass for quantification of metals.

2.6 - Data analysis

Growth and emergency data from Jun1, Jun2, Aug1, Mar and Apr1 experiments were submitted to Mann Whitney U test, while those from Aug2 and Apr2 were submitted to Kruskal-Wallis with Dunn’s post hoc. Analyses were carried out in the Past program (HAMMER *et al*., 2001). In order to verify differences in the survival, Fisher's exact test was conducted in the TOXSTAT 3.4 program (WEST Inc. and GULLEY, 1996).

Avoidance responses were evaluated according to ISO (2008), using the formula:

$$X = \left(\frac{n_c - n_t}{N}\right) \times 100$$

Where: X = avoidance, expressed as a percentage;

n_c = number of organisms in control;

n_t = number of organisms in the sample;

N = total number of organisms exposed.

There is no sample avoidance when -20 < x < 20, and sample avoidance when 20 < x < 100. When -100 < x < -20, there is control avoidance.

Bioaccumulation factors (BAF) were calculated by the ratio of the concentration of metal in the tissue of organisms (C_{org}) and the concentration of metal in the sediment (C_{sed}):

$$BAF = C_{org}/C_{sed}$$

Principal component analysis (PCA) was used to evaluate the relationship between bioaccumulation in *C. xanthus* exposed to the laboratory control sediment and sediment samples from P5, and in organisms from P4.

3 – Results

3.1 - Samples characterization

Most part of metals and metalloid concentrations in the environmental samples (Table 1) were above the limits established by Brazilian legislation (CONAMA Resolution nº 454/12 - BRASIL, 2012). The organic matter content was characteristic of mineral sediment (<10%), which is mainly composed of silica, clay, calcium, iron and manganese compounds (UNGEMACH, 1960). The sediment granulometry, was composed of larger fractions of fine sand and silt.

Although it was detected the presence of As, Cu, Zn, Al, Fe, Mg and Mn in the laboratory control sediment, their concentrations were all below the limits of the legislation. This sediment comes from a protected area, so the presence of metals such as Al and Fe, is due to the natural constitution of the soils of the region.

Table 1 – Concentration of metals and metalloid ($mg.kg^{-1}$), granulometry (%) and organic matter (%) in sediments and limits established by CONAMA Resolution nº 454/12 (BRASIL, 2012) for levels 1 and 2.

	Control	P5				P4		Levels	
	Sediment	Jun/17	Aug/17	Mar/18	Apr/18	Mar/18	Apr/18	1*	2**
Arsenic (As)	0.12	2597.31	2301.92	582.14	505.34	240.50	448.75	5.9	17.0
Cadmium (Cd)	ND	40.19	39.15	ND	ND	ND	ND	0.6	3.5
Lead (Pb)	ND	20.51	22.28	7.20	23.58	18.63	ND	35.0	91.3
Copper (Cu)	8.05	497.12	885.58	309.02	137.65	140.19	276.83	35.7	197.0
Chromium (Cr)	ND	117.69	187.50	103.14	161.86	127.88	105.48	37.3	90.0
Nickel (Ni)	ND	70.19	108.17	24.74	56.27	40.87	29.01	18.0	35.9
Zinc (Zn)	8.11	183.27	328.27	80.98	71.27	76.54	46.18	123.0	315.0
Aluminum (Al)	1528.70	7990.38	12750.00	3702.94	8931.37	9451.92	4120.19	-	-
Iron (Fe)	1065.74	109038.50	96634.62	53431.40	83039.20	86634.62	51538.46	-	-
Magnesium (Mg)	80.37	3924.04	2342.31	1237.25	2166.67	1306.73	1136.54	-	-
Manganese (Mn)	3.57	1098.08	982.69	95980.40	896.08	874.04	55576.92	-	-
Granulometric fractions (%)									
Coarse Sand		1.40	3.92	5.57	2.88	7.00	7.60		
Fine Sand		46.50	40.47	44.06	44.26	49.90	44.20		
Silt		50.10	53.97	48.86	50.57	35.10	40.90		
Clay		2.00	1.65	1.51	2.29	8.00	7.30		
Organic Matter (%)		7.83	11.87	7.91	6.37	10.50	7.82		

ND: the concentration was below the limit of detection.
*: Level 1: threshold, below which there is low probability of adverse effects to biota.
**: Level 2: threshold, above which there is high probability of adverse effects to biota.

3.2 - Partial life cycle experiments

Growth (dry weight) in eight days of larvae exposed to the sediment samples from P5 was significantly smaller than controls in all experiments (Figure 2), except for the first generation in April. There was no significantly effect on survival of larvae exposed to environmental samples across eight days in any experiment ($p > 0.05$) (Table 2).

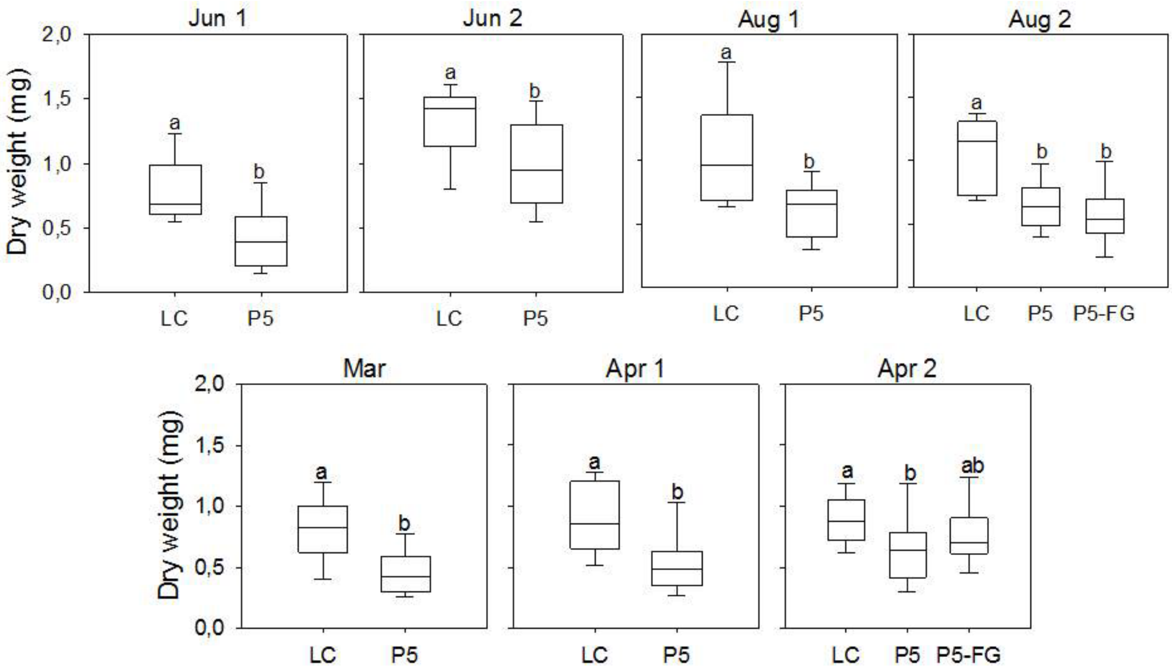

Figure 2 – Effects of sediment samples from P5 on *C. xanthus* larvae growth. LC = Laboratory control; P5-FG = 1st generation exposed to environmental sample. $p < 0.05$.

There was a significant difference in number of adults emerged in sediment samples from P5 compared to controls in the first experiment of the dry season and in all of the rainy season (Table 2). Regarding the time of emergency, adults in the controls emerged at least one day before the ones of the environmental samples, with statistically significant difference, in all experiments, except for the first generation in April. Although this first generation did not present growth reduction and delayed emergence, it presented significant mortality at the end of the test.

Percentage of survival (>70%) and emergence of adults (>65%) in the laboratory controls confirmed the validity of the experiments, according to USEPA (2000).

Table 2 – Parameters evaluated in *C. xanthus* partial life cycle experiments with sediment samples from P5.

Parameters	Experiment	Laboratory Control	P5	P5 - 1st generation
Survival in 8 days (%)	Jun1	100	90	--
	Jun2	100	90	--
	Aug1	100	100	--
	Aug2	100	100	100
	Mar	100	100	--
	Apr1	100	95	--
	Apr2	100	100	80
Initial emergency (days)	Jun1	11	13	--
	Jun2	10	12	--
	Aug1	10	12	--
	Aug2	10	11	12
	Mar	9	10	--
	Apr1	10	11	--
	Apr2	10	11	10
Emergency peak (days)	Jun1	13 ± 1.2	15 ± 0.8*	--
	Jun2	12 ± 0.9	14 ± 0.8*	--
	Aug1	12 ± 0.9	13 ± 1.1*	--
	Aug2	12 ± 0.9	13 ± 1.2*	13 ± 1.0*
	Mar	10 ± 0.4	11 ± 1.4*	--
	Apr1	11 ± 0.7	12 ± 1.2*	--
	Apr2	11 ± 0.5	12 ± 0.8*	12 ± 0.9
Emergency (%)	Jun1	82	40**	--
	Jun2	95	90	--
	Aug1	88	85	--
	Aug2	98	75	78
	Mar	100	68**	--
	Apr1	90	48**	--
	Apr2	100	80**	63**

Significant difference compared to the laboratory control: * $p \leq 0.001$; ** $p \leq 0.05$; $n = 40$.

3.3 - Avoidance experiments

The larvae behavior evaluation showed no environmental sample avoidance according to ISO's formula (Table 3). In the tests with environmental sample and control sediments, control avoidance was verified in the June and March campaigns, that is, organisms preferred the environmental sample. In the other experiments, there was no avoidance response. In the dual tests, with control sediment and environmental sample on both sides, the organisms presented homogeneous distribution.

Table 3 – Avoidance response of *C. xanthus* exposed to sediment samples from P5 and control sediments.

	X %				Effect			
	Jun	Aug	Mar	Apr	Jun	Aug	Mar	Apr
Environmental Sample + Control	- 40	- 11	- 45	- 17	Control avoidance	No	Control avoidance	No
Dual Laboratory Control	7	0	0	0	No	No	No	No
Dual Environmental Sample	- 10	12	10	- 3	No	No	No	No

3.4 - Bioaccumulation

The concentrations of metals and metalloid quantified in the tissues proved that *C. xanthus* is capable of bioaccumulating these elements (Table 4). The larvae exposed to the P5 samples had concentrations of the same order of magnitude as those obtained in the organisms collected in P4. The sample of P4 in March presented higher biomass of Oligochaeta, while the sample in April showed higher biomass of Chironomidae.

BAFs were not calculated for the metals that were below the detection limits in the sediments. The BAFs showed variability between campaigns and in general were lower than 1, i.e. the tissue concentrations were lower than in the sediments.

Table 4 – Bioaccumulation ($mg.kg^{-1}$) and bioaccumulation factors (BAF) of metals and metalloid in *C. xanthus* (Control and P5) and in benthic macroinvertebrates from P4.

	Control		P5				P4			
	Mar	Apr	Mar	Apr	BAF_{Mar}	BAF_{Apr}	Mar	Apr	BAF_{Mar}	BAF_{Apr}
As	0.76	0.38	138.03	196.40	0.24	0.39	266.78	152.29	1.11	0.34
Cd	ND	ND	ND	ND	--	--	ND	ND	--	--
Pb	ND	ND	1.35	1.63	0.19	0.07	3.34	2.24	0.18	--
Cu	16.46	16.22	218.03	330.06	0.71	2.40	368.67	192.92	2.63	0.70
Cr	2.60	2.50	33.04	40.42	0.32	0.25	10.55	33.75	0.08	0.32
Ni	0.53	0.24	17.94	21.06	0.73	0.37	1.89	15.63	0.05	0.54
Zn	464.52	375.29	158.02	126.60	1.95	1.78	487.25	210.66	6.37	4.56
Al	237.97	309.53	2864.37	3205.78	0.77	0.36	1370.83	2260.19	0.15	0.55
Fe	627.00	615.63	11706.67	14434.83	0.22	0.17	3348.88	14480.66	0.04	0.28
Mg	1425.04	1269.65	1402.02	1500.78	1.13	0.69	1387.79	1429.69	1.06	1.26
Mn	11.64	10.25	298.33	356.26	0.003	0.40	36.46	141.39	0.04	--

ND: the concentration was below the limit of detection.

The PCA analysis (Figure 3) explained 92.24% of the variability in the two first components (PC1: 71.35%, PC2: 20.89%). PC1 was mainly correlated with the metals Cr, Ni, Al, Fe, Mg and Mn, while PC2 was positively correlated with the elements As, Pb, Cu and Zn. The bioaccumulation of *C. xanthus* exposed to the sample from P5 in March and April and the organisms collected in P4 in March were positively correlated with PC1, while the organisms collected in P4 in April was positively correlated with PC2. The controls were negatively correlated with the two axes.

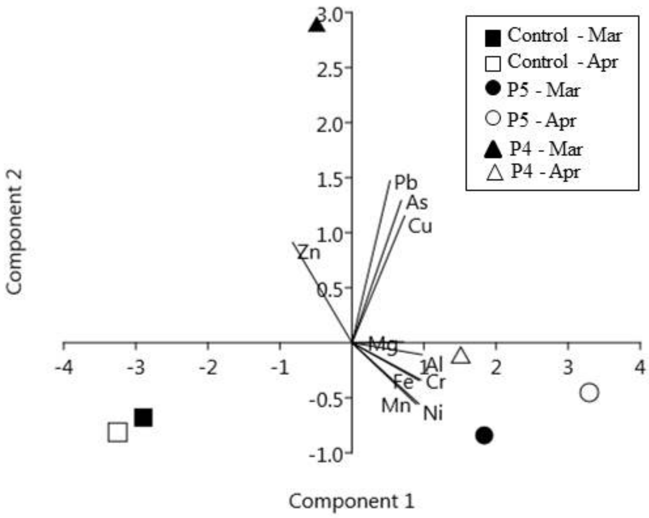

Figure 3 – Principal component analysis of metal and metalloid concentrations in the tissues of organisms.

4 – Discussion

In the environmental samples, in general, all metals have exceeded the limits of the legislation at least once, with the exception of Pb, which has always been below level 1. Only As, Cd and Zn showed evident seasonal variation, with lower concentrations in the rainy season. High concentrations of Al, Fe, Mn and As are characteristic of the study area, belonging to the Iron Quadrangle of Minas Gerais, with soils rich in iron ore, manganese oxides and gold ore that is rich in arsenic (BORBA *et al.*, 2000; FILHO *et al.*, 2011). The sediments granulometry, composed of larger fractions of fine sand and silt, influences the high concentrations found, since they can adsorb high contents of metals (SOUZA, 2015).

Although the high concentrations of metals in sediments indicate adverse effects on the biota, preliminary experiments showed no mortality in acute toxicity tests with *C. xanthus* (SALES, 2013). The use of larvae mortality as endpoint does not always encompass the ecological effects of contaminants (MOGREN and TRUMBLE, 2010), which was confirmed by the bioaccumulation and effects on emergency verified in these partial life cycle experiments.

Studies have shown that larval growth correlates with the amount of food available (SIBLEY *et al*., 1997; PÉRY *et al*., 2002) and bioaccumulation of metals has been related to growth decrease in chironomids larvae (TIMMERMANS *et al*., 1992; MILANI *et al.,* 2003; PÉRY *et al*., 2005). Although the environmental sample contained more organic matter than control (calcinated sand), larval growth impairment in samples can be explained by the sublethal toxic effect of metals.

Growth impairment caused by metal contamination has been verified in chironomids (FARIA *et al*., 2006; FARIA *et al*., 2007; CHIBUNDA *et al*., 2008). Growth was highly inhibited in *C. riparius* after *in situ* exposure to metal contaminated rivers, suggesting that this is a sensitive and appropriate tool to be used as a bioindicator of metal pollution (FARIA *et al*., 2006). *C. riparius* body length increase was significantly inhibited by metal contamination from an abandoned goldmine, both *in situ* and laboratory bioassays (FARIA *et al*., 2007). Thus, laboratory bioassays can provide a reliable indication of the toxicity in areas contaminated by metals.

In the present study, development time was also affected, with delay at beginning and peaks of emergency compared to the controls. A similar result was obtained for *C. riparius* exposed to contaminated sediment from a gold mining area (CHIBUNDA *et al*., 2008). Adult emergence time has been considered the most sensitive endpoint for *C. riparius* exposed to metals, with delay in emergency time in concentrations of Cu, Cd and Hg that did not affect growth (CHIBUNDA, 2009; MARINKOVIĆ *et al*., 2011; MARINKOVIĆ *et al*., 2012a).

The mortality effect was not a good endpoint due to the variability between campaigns. Although mortality due to metals was verified in chironomids exposed to environmental samples (QI *et al*., 2015), Marinković *et al*. (2012b) observed variability in mortality in multi-generation experiments.

The ability of *C. xanthus* as well as of the native organisms sampled to bioaccumulate metals, has also been shown, evidencing the impacts generated by local mining activity. The difference in the biomass of the groups found in P2 in the two campaigns may have influenced the variability in bioaccumulation at this site. In the April samples, when higher biomass of chironomids was obtained, the concentrations were closer to those obtained for *C. xanthus* exposed to the P1 samples. PCA demonstrated the greater proximity of the responses of these organisms. Thus, *C. xanthus* may have reflected the effects on native organisms.

Studies have shown differences in sensitivity (MILANI *et al*., 2003) and variability in the bioaccumulation of metals between oligochaetes and chironomids. For organisms collected in the field, Arslan *et al*. (2010) observed that oligochaetes accumulated more Pb, with no differences for Cd, Cr, Zn, while chironomids accumulated more Cu and Ni. Bervoets *et al*. (1997) observed higher accumulation of Cu, Cd and Pb by oligochaetes and a similar value for Zn. Differences in bioaccumulation have also been found among benthic organisms of different guilds (CORBI *et al*., 2010, LI *et al*., 2016) and trophic levels (KALANTZI *et al*., 2014; HAMIDIAN *et al*., 2016).

Variations in bioaccumulation responses between metals were also observed within chironomid species (DESROSIERS *et al*., 2008; HAMIDIAN *et al.,* 2016; KEMP *et al*., 2017). The potential of bioaccumulation by chironomids has been related to physiological mechanisms (POSTMA and DAVIDS, 1995) and factors such as sediment grain size and organic matter content (DESROSIERS *et al*., 2008). The high metal tolerance in Chironomidae is related to its ability to regulate the concentration of some metals such as Cu, Zn, Cd, Pb, Ni, Mn (KRANTZBERG and STOKES, 1989; TIMMERMANS *et al*., 1992), which may be related to the absence of acute toxicity in this study.

The PCA showed that the bioaccumulation by *C. xanthus* exposed to the environmental sample of P1 was negatively correlated with As, Pb, Cu and Zn, suggesting that *C. xanthus* was able to regulate the accumulation of these elements. While regulating the concentration of essential elements such as Cu and Zn is already expected, the regulation of As, Pb and Cd by chironomids has been suggested in the literature (HAMIDIAN *et al*., 2016).

For control sediments, the calculation of BAFs is not appropriate, since low concentrations of contaminants and/or organic carbon result in high BAFs. BAFs are not independent of the concentrations of contaminants in the sediments and, therefore, it is not a direct measure of toxicity. On the other hand, sediments with high concentrations of contaminants can generate low BAFs due to factors such as the maximum that can be accumulated in the body, altered metabolism by the degree of contamination and sediment characteristics that affect the contaminants bioavailability (DESROSIERS *et al.,* 2008; VAN GEEST and WATSON-LEUNG, 2016). Taking it into account, the BAFs obtained in the present study can be used for comparisons over time and between samples from other sites, providing data for evaluations of the relationship between grain size, organic matter content and species.

With respect to behavior, *C. xanthus* larvae did not show avoidance of environmental sediment samples. Avoidance behavior of contaminated sediment was also evaluated for *C.*

riparius (DORNFELD *et al*., 2009) and *C. salinarius* (HARE and SHOONER, 1995) exposed to Cu and Cd, respectively, which showed no preference for control. On the other hand, *C. tentans* exposed to sediments of lakes with different concentrations of Cd, Zn and Cr showed avoidance of sediments with extremely high metals concentrations (> 422 $mg.kg^{-1}$ Cd, > 8330 $mg.kg^{-1}$ Zn, > 1513 $mg.kg^{-1}$ Cr) (WENTSEL *et al*., 1977).

De Hass *et al*. (2006) observed that *C. riparius* has a high preference for sediments with high food quality, independent of contamination level. There was avoidance of contaminated sediment only when the difference in contaminant concentration was high and the difference in food quality was low. The authors concluded that *C. riparius* is able to identify differences between sediments and its selection is more influenced by food quality than by the presence of contaminants. Thus, considering the high tolerance of chironomids and the chronic effects observed, avoidance experiments have not shown to be a good tool to evaluate sediments contaminated by metals.

Studies suggest that chronic exposure of chironomids to high concentrations of metals may lead to the development of metal tolerance (KRANTZBERG and STOKES, 1989), which is due to its phenotypic plasticity (MARINKOVIĆ *et al*., 2012b). However, sublethal effects of chronic exposure to metals have already been observed at the level of somatic changes in the chromosomes of different species of Chironomidae in the same habitat, with different levels of responses between species (SZAREK-GWIAZDA *et al*., 2013). Marinković *et al*. (2012a) found that low concentrations of Cu and Cd, which did not affect larval growth, had a profound impact on gene expression, with changes in several transcripts indicative of general stress.

Chironomids have been found across the studied area. The streams nearby were considered highly degraded by anthropic action, presenting dominance of chironomids and oligochaetes (MORENO *et al*., 2010, FEIO *et al.,* 2015). Considering that high concentrations of As, Cd, Cu, Ni, Cr and Zn have been already found in sediments of the Iron Quadrangle (VICQ *et al*., 2015), these organisms have been subjected to the effects of chronic toxicity of the metals being bioaccumulated. Nevertheless, the ecotoxicological approach of sediments is not contemplated in the Brazilian environmental legislation.

Considering the difference in sensitivity between species, it is important to reinforce the use of native organisms to evaluate environmental contamination. Many ecotoxicological studies have used organisms and test protocols from temperate regions, which have been pointed out as not suitable to evaluate the effects of toxicants in tropical and sub-tropical countries (KWOK *et al*., 2007; DAAM and VAN DEN BRINK, 2010; RIETZLER *et al.,* 2017).

C. xanthus has been indicated as test organism in ecotoxicological studies in tropical regions (RIETZLER *et al.,* 2017). Alves and Rietzler (2015) observed acute toxicity on them, when exposed to sediment samples contaminated by chromium-rich industrial effluents, as well as deformities in the mentum of chironomids specimens collected in the field, demonstrating the effectiveness of the ecotoxicological approach in the environmental forensic investigation of areas suspected of contamination by tanneries.

Although sediment acute toxicity effects on chironomids are in general not observed, chronic exposure shows that they are able to bioaccumulate and can have its development impaired by high concentrations of metals, and even a decrease in the number of emerging adults. Thus, the sublethal toxic effects observed provide consistent results for use as evidence for environmental forensic investigation in mining areas.

The experiments demonstrated that *C. xanthus* can be a good tool to be used in monitoring and evaluation of sediments contaminated by metals. They can also provide useful information for forensic investigation. Therefore, *C. xanthus* should be standardized as test organism.

5 – Conclusions

Slower growth, delayed emergency and bioaccumulation of metals in *C. xanthus* larvae evidenced the toxic effects of sediments from gold processing areas on benthic macroinvertebrates. *C. xanthus* has shown to be a good indicator of metal contamination.

The quantification of metals in benthic macroinvertebrates from the field reinforced ecological risk in the Iron Quadrangle.

Avoidance experiments did not prove to be a good tool to evaluate behavioral effects of toxicity in *C. xanthus*. However, other sediment toxicity tests, mainly related to sublethal effects, could provide useful tools for environmental monitoring and legislation procedures for pollution and contamination control. Therefore, *C. xanthus* should be standardized as test organism and the ecotoxicological assessment of sediments should be included in the Brazilian environmental legislation.

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7 - CONSIDERAÇÕES FINAIS

A avaliação ecotoxicológica realizada neste estudo possibilitou uma visão mais completa da qualidade ambiental no alto curso da sub-bacia do rio das Velhas, na bacia do rio São Francisco.

Os ensaios ecotoxicológicos com amostras de água demonstraram efeitos tóxicos em pontos onde as concentrações de metais e arsênio estiveram abaixo dos limites da legislação. Nestes locais, o monitoramento realizado pelo IGAM utiliza apenas parâmetros físicos e químicos, considerando-os com boa qualidade. Verificou-se assim, que a ecotoxicidade é um importante parâmetro de avaliação da qualidade dos cursos de água, que deve ser implantado em mais pontos de monitoramento ambiental em áreas de mineração.

Na avaliação dos sedimentos, foram quantificadas altas concentrações de arsênio em todos os pontos de amostragem e de diversos metais nos pontos da área de influência direta da atividade de beneficiamento de ouro. Embora tenha havido ausência de toxicidade aguda a *C. xanthus* em todos os pontos, verificou-se toxicidade crônica recorrente a *C. silvestrii* e *C. dubia* expostas às amostras de sedimento total de todos os pontos, enquanto os elutriatos causaram toxicidade aguda e/ou crônica aos cladóceros.

Além disso, a condução de ensaios de toxicidade com o ciclo de vida parcial de *C. xanthus* expostos às amostras de sedimento do ponto mais próximo da bacia de contenção da atividade de beneficiamento de ouro, mostrou efeitos tóxicos relacionados ao menor crescimento das larvas e emergência de adultos. Os ensaios de toxicidade tendo a biomassa como parâmetro de avaliação, mostraram-se de fácil condução, com respostas rápidas e de baixo custo. Verificou-se ainda bioacumulação de metais e arsênio nas larvas expostas a estes sedimentos em laboratório, bem como em macroinvertebrados bentônicos coletados em um ponto a jusante. Neste contexto, pode-se verificar o efeito da exposição de organismos bentônicos em áreas de mineração e o bom desempenho de *C. xanthus* como bioindicador dos efeitos crônicos de sedimentos.

Embora a legislação brasileira não contemple a avaliação ecotoxicológica dos sedimentos no monitoramento dos cursos de água superficiais, a legislação estadual de Minas Gerais, DN COPAM/CERH-MG nº01/08, estabelece a "não verificação de efeito tóxico agudo e crônico a organismos em amostras de água e/ou sedimento", para rios classe 1 e 2. Os dados obtidos neste estudo demonstraram, a necessidade de se incluir a avaliação química, física e ecotoxicológica dos sedimentos no monitoramento dos cursos de água de Minas Gerais, tendo em vista seu papel conservativo e de fonte de contaminantes.

Os bioensaios com os elutriatos dos solos revelaram efeitos tóxicos aos cladóceros, demonstrando a baixa capacidade de retenção e o potencial como fonte de contaminantes dos mesmos, especialmente nos locais de influência direta da mineração de ferro e do beneficiamento de ouro. Portanto, a avaliação da toxicidade dos solos deve ser incluída na legislação ambiental e conduzida no monitoramento ambiental do estado, além disso, ações visando a recuperação de áreas degradadas devem ser implementadas, visto que estudos já demonstraram efeitos tóxicos dos solos de áreas de mineração à fauna edáfica e a acumulação de arsênio em moradores da região onde há a atividade de beneficiamento de ouro.

A verificação de efeitos de toxicidade aguda da água tanto a *Daphnia similis* quanto *D. laevis* e as diferenças entre *Daphnia* spp. e *Ceriodaphnia* spp. confirmaram a importância de se implantar mais de um tipo de ensaio ecotoxicológico, a fim de melhor respaldar o monitoramento ambiental de Minas Gerais.

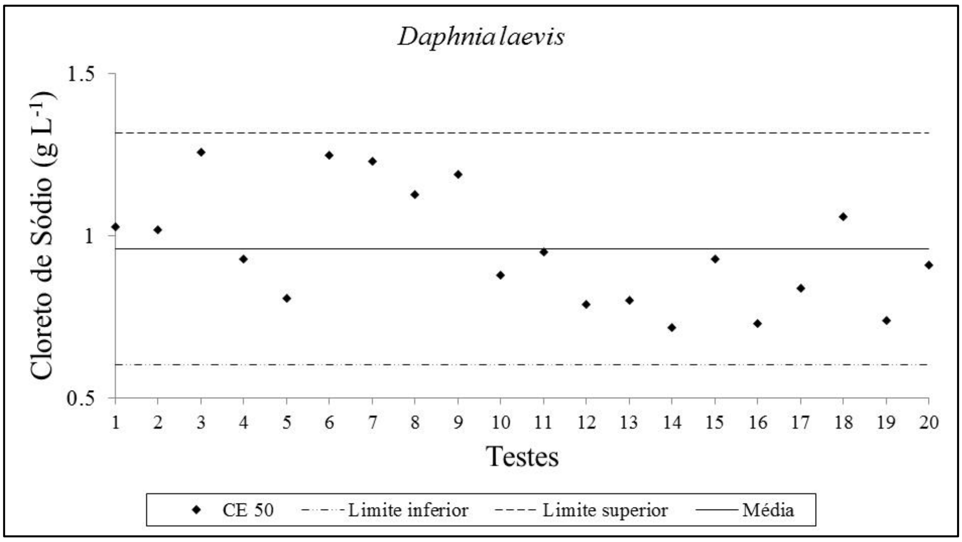
Tendo em vista que as diferenças de respostas entre *D. laevis* e *D. similis* entre os pontos de amostragem, onde *D. laevis* mostrou-se mais sensível para a água dos pontos mais contaminados e que *C. silvestrii* mostrou-se tão sensível quanto *C. dubia* para água superficial e com diferenças de sensibilidade para os elutriatos, demonstrou-se a importância e a possibilidade da utilização de espécies nativas, para uma avaliação mais representativa dos efeitos da poluição à biota local. Além disso, estudos com organismos nativos têm demonstrado resultados satisfatórios em relação ao cultivo e em testes de sensibilidade. Não se justifica, assim, o uso de *C. dubia* no monitoramento ambiental, sendo *C. silvestrii*, espécie nativa, já padronizada e amplamente utilizada em estudos ecotoxicológicos. Além disso, *C. xanthus* apresentou desempenho comparável aos seus congêneres de região temperada em estudos em áreas de mineração, demonstrando potencial para padronização como bioindicador em testes com sedimentos.

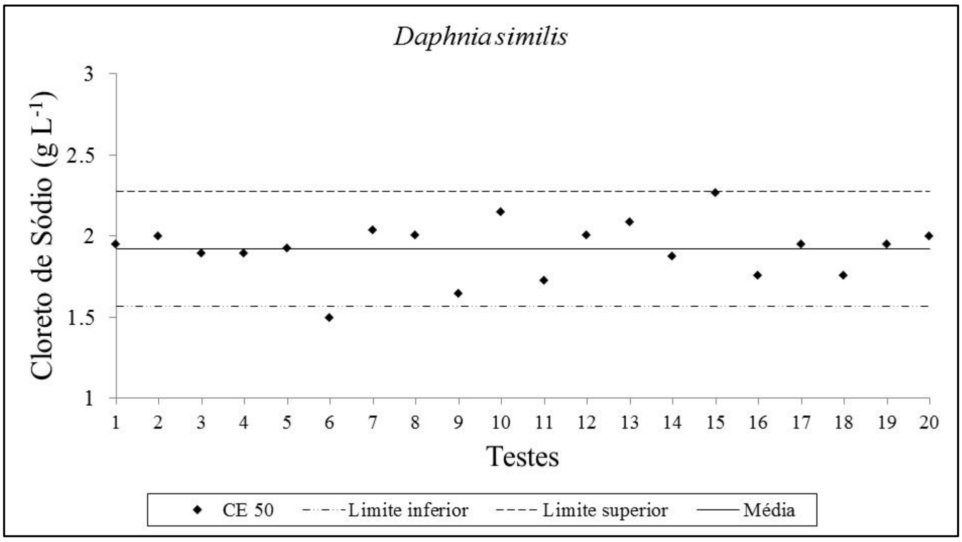
A Lei n° 9605/1998 (Brasil, 1998) define como crime de poluição, no Artigo 54, "*Causar poluição de qualquer natureza em níveis tais que resultem ou possam resultar em danos à saúde humana, ou que provoquem a mortandade de animais ou a destruição significativa da flora*". Do ponto de vista da perícia ambiental, os ensaios ecotoxicológicos com cladóceros, tanto com amostras de água como de sedimentos dos pontos onde houve mortalidade, demonstraram ser uma ferramenta eficiente na comprovação dos crimes de poluição em áreas sob influência direta de atividades minerárias. Já os ensaios de toxicidade crônica com *C. xanthus*, embora não tenham mostrado efeito recorrente de mortalidade, demonstraram potencial para uso na perícia e para tanto, é necessário que esta espécie seja padronizada para garantir a confiabilidade dos resultados como provas em casos de poluição.

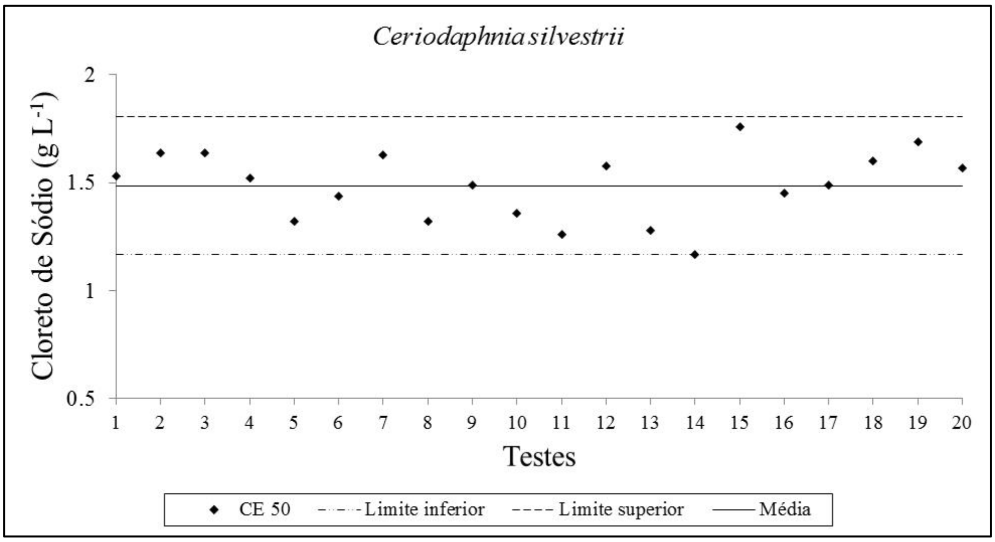
Neste contexto, a abordagem ecotoxicológica mostrou-se um instrumento aplicável para o monitoramento e construção de provas em casos de perícia ambiental em áreas de mineração.

APÊNDICE

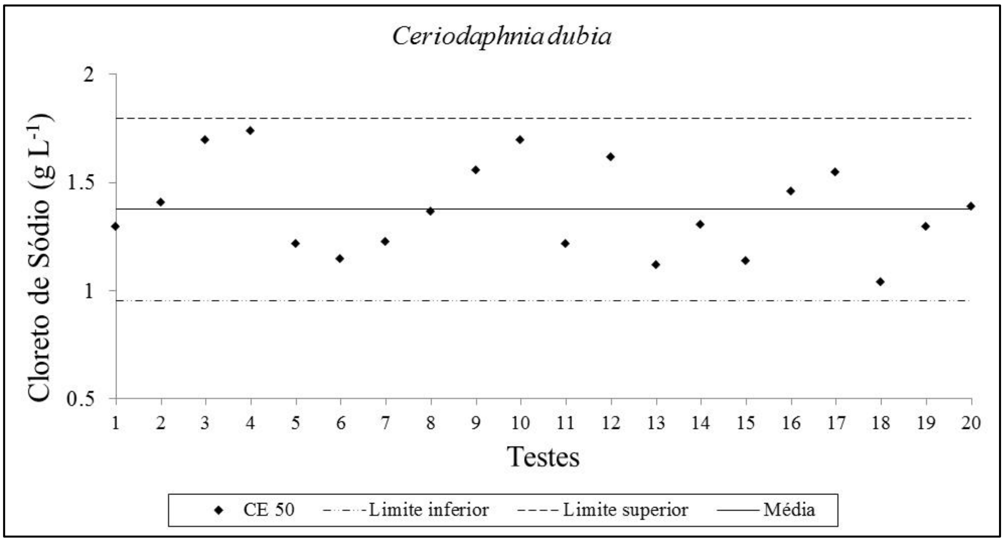
Cartas-controle de sensibilidade dos organismos-teste utilizados no estudo.



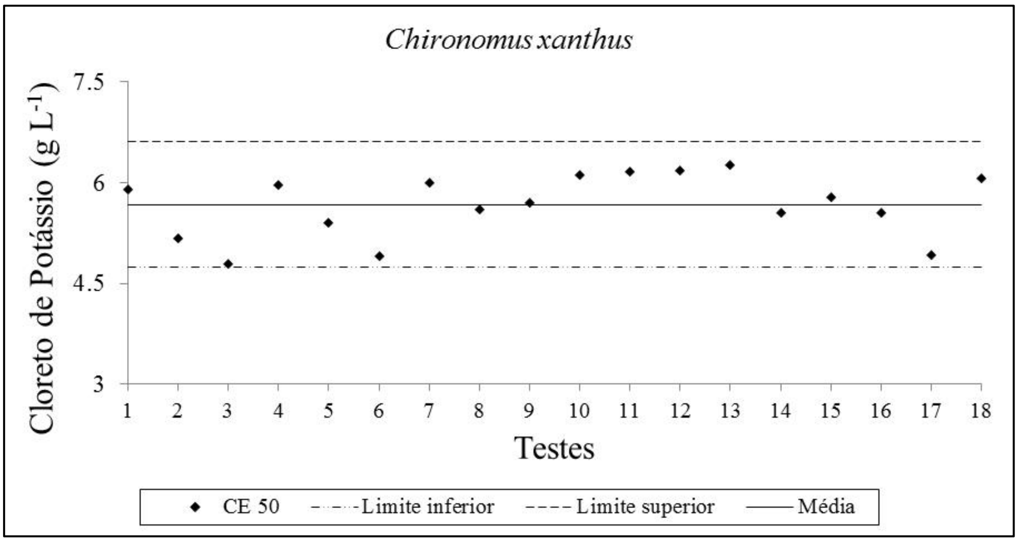




Ceriodaphnia silvestrii
Cloreto de Sódio (g L-1)
2
1.5
1
0.5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Testes
CE 50
Limite inferior
Limite superior
Média



Ceriodaphnia dubia
Cloreto de Sódio (g L-1)
2
1.5
1
0.5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Testes
CE 50
Limite inferior
Limite superior
Média



Chironomus xanthus
Cloreto de Potássio (g L-1)
7.5
6
4.5
3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Testes
CE 50
Limite inferior
Limite superior
Média

Fotos dos pontos de amostragem

P1:

P2:

P3:

P4:

P5:

P6: