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MARCELINO BENVINDO DE SOUZA

**Efeito da paisagem transformada na frequência de danos
genotóxico e mutagênico em morcegos**

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MARCELINO BENVINDO DE SOUZA

Efeito da paisagem transformada na frequência de danos genotóxico e mutagênico em morcegos

Tese apresentada ao Programa de Pós-Graduação em Ciências Ambientais da Universidade Federal de Goiás - UFG, como requisito para obtenção do título de Doutor em Ciências Ambientais.

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ATA DE DEFESA DE TESE

Ata N° 001/2023 da sessão de Defesa de Tese de **Marcelino Benvindo de Souza** que confere o título de Doutor em **Ciências Ambientais**, na área de concentração em **Estrutura e Dinâmica Ambiental**.

Aos treze dias do mês de janeiro do ano de 2023, a partir das 14h, na sala virtual da plataforma Google Meet: meet.google.com/kga-xsxw-cmw, realizou-se a sessão pública de Defesa de Tese intitulada "Efeito da paisagem transformada na frequência de danos genotóxico e mutagênico em morcegos". Os trabalhos foram instalados pela Orientadora, Professora Doutora **Daniela de Melo e Silva** (ICB/UFG), com a participação dos demais membros da Banca Examinadora: Professora Doutora **Karla Maria Silva de Faria** (IESA/UFG), membro titular interno; Professor Doutor **Klebber Teodomiro Martins Formiga** (EECA/UFG), membro titular interno, Professor Doutor **Thiago Bernardi Vieira** (UFPA), membro titular externo; Professor Doutor **Vinicius Guerra Batista** (UFMT), membro titular externo. Durante a arguição os membros da banca **não** sugeriram alteração do título da tese. A Banca Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Tese tendo sido o candidato **sido aprovado** pelos seus membros. Proclamados os resultados pela Professora Doutora **Daniela de Melo e Silva**, Presidente da Banca Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Banca Examinadora, aos treze dias do mês de janeiro do ano de 2023.

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RESUMO

O impacto das mudanças na paisagem natural, como a perda e fragmentação de habitats resultantes da expansão agrícola e mineração, representam significativas ameaças à conservação da biodiversidade. Diante desse cenário, o uso de espécies bioindicadoras como os morcegos é indicado para lançar luz da saúde ambiental. Embora ocorram em uma ampla faixa geográfica, são vulneráveis a uma série de estressores, principalmente químicos, como pesticidas e metais tóxicos que podem ser obtidos por meio dos alimentos contaminados, água e contato dérmico. No primeiro Capítulo da tese avaliamos a tendência da produção científica sobre morcegos e poluição no Brasil no contexto da ecotoxicologia. Os resultados da revisão indicaram uma escassez de estudos publicados até Setembro de 2019, cuja ênfase era principalmente voltada a pesticidas e metais pesados como estressores ambientais. Em seguida estudos *in situ* foram conduzidos no cerrado Goiano. Em áreas de lavouras de soja e cana-de-açúcar versus unidade de conservação, morcegos foram investigados para dano genotóxico e mutagênico. Os resultados indicaram que morcegos de áreas agrícolas apresentaram maior frequência de danos genotóxicos quando comparados àqueles da área conservada. Concomitantemente avaliamos a sensibilidade dos morcegos para a freqüência de dano genotóxico e mutagênico em áreas de mineração a céu aberto. Os resultados reforçam a sensibilidade dos morcegos em paisagem transformada pela mineração, cujas lesões no DNA podem estar associadas à exposição aos metais. Esses estudos concluíram que algumas espécies de morcegos são sensíveis à perturbação ambiental, e biomarcadores menos invasivos e de baixo custo como o ensaio cometa e o teste de micronúcleo podem contribuir para análise da saúde ambiental. Finalmente, um trabalho em anexo foi incluído na tese onde descreve a composição de espécies de morcegos em áreas de degradação crônica da paisagem em plantações de soja e cana-de-açúcar. No estudo, são apresentadas 18 espécies de morcegos, pertencentes a três famílias, Phyllostomidae (88%), Molossidae (8%) e Vespertilionidae (4%). Em resumo, esses estudos fortalecem a compreensão das espécies de morcegos do cerrado goiano e sua suscetibilidade à degradação do cerrado.

Palavras-chave: ensaio cometa; teste do micronúcleo; mucosa bucal; morcegos; agricultura; mineração.

ABSTRACT

The impact of changes in the natural landscape, such as habitat loss and fragmentation resulting from agricultural expansion and mining, could cause significant threats to biodiversity conservation. Given this scenario, bioindicator species such as bats are indicated to shed light on environmental health. Although they occur in a wide geographic range, they are vulnerable to various stressors, mainly chemicals, such as pesticides and toxic metals that can be obtained through contaminated food, water, and dermal contact. In the first chapter of the thesis, we evaluated the tendency of scientific production on bats and pollution in Brazil in the context of ecotoxicology. The review results indicated a scarcity of studies published until September 2019, whose emphasis was mainly on pesticides and heavy metals as environmental stressors. Then in situ studies were conducted in the cerrado of Goiás. In areas of soybean and sugarcane crops versus protected areas, bats were investigated for genotoxic and mutagenic damage. The results indicated that bats from agricultural areas showed a higher frequency of genotoxic damage when compared to those from the conserved area. Concomitantly, we evaluated the sensitivity of bats to the frequency of genotoxic and mutagenic damage in open pit mining areas. The results reinforce the sensitivity of bats in a landscape transformed by mining, whose DNA damage may be associated with exposure to metals. These studies concluded that some species of bats are sensitive to environmental disturbance and less invasive and low-cost biomarkers such as the comet assay and the micronucleus assay can contribute to the analysis of environmental health. Finally, an attached work was included in the thesis where it describes the species composition of bats in areas of chronic landscape degradation in soybean and sugar cane plantations. In the study, 18 species of bats are presented, belonging to three families, Phyllostomidae (88%), Molossidae (8%), and Vespertilionidae (4%). In summary, this study strengthens the understanding of bat species in the cerrado of Goiás and their susceptibility to degradation of the cerrado.

Keywords: comet assay; micronucleus test; oral mucosa; bats; agriculture; mining.

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LISTADEABREVIATURASESIGLAS

DNA - Ácido desoxirribonucléico
MN - Micronúcleo
ANs - Anormalidades Nucleares
Cr - Cromo
Mn - Manganês
Fe - Ferro
Ni - Níquel
Cu - Cobre
Zn - Zinco
Cd - Cádmio
Pb - Chumbo
Al - Alumínio
Si - Silício
Ba - Bário
Hg - Mercúrio
ENP - Emas National Park (Parque Nacional das Emas)
PNE - Parque Nacional das Emas
So - Soja
SC - Cana-de-açúcar
ICMBio - Instituto Chico Mendes de Conservação da Biodiversidade
S - Sítio
n - Número
DDT - Dicloro-Difenil-Tricloroetano
CONAMA - Conselho Nacional do Meio Ambiente
 μL - Microlitro
ng - Nanograma
mg/L - Miligrama por litro
 $^{\circ}\text{C}$ - Graus Celsius
DMSO - Dimetilsulfóxido
nm - Nanômetro
TL - Tail length
% DNA - Percentage of DNA in the tail
OTM - Olive Tail Moment
NaCl - Cloreto de Sódio
ANOVA - Análise de Variância
F - Anova (Análise de Variância)
t - Teste t de Student
r - Correlação
H - Kruskal-Wallis
U - Mann-Whitney

1. Introdução Geral

Mais de 77% da cobertura do solo da Terra foi afetada pelas atividades humanas, o que reduziu e fragmentou os habitats adequados para a vida selvagem (KUIPERS et al., 2021). Muitas atividades como a agricultura, pecuária, urbanização, mineração e usinas hidrelétricas estão entre aquelas que geram grandes mudanças na paisagem natural. Tendo em vista que as modificações no uso da terra são a maior ameaça à biodiversidade em escala global, a agricultura tem sido a principal impulsionadora dessas mudanças com aproximadamente 40% da cobertura do solo terrestre (FRICK et al., 2020; BRAZEIRO et al., 2020).

No Brasil, por exemplo, a produção agrícola é favorecida pelas condições climáticas e pela grande quantidade de terras disponíveis o que torna nosso país o segundo maior exportador de produtos agrícolas do mundo (PAUMGARTTEN, 2020; BROVINI et al., 2021). Em termos de seguimentos agrícolas, o Brasil é o segundo maior produtor de soja (PAUMGARTTEN, 2020) e o maior produtor de cana-de-açúcar do mundo (ACAYABA et al., 2021).

Para assegurar o desenvolvimento da agricultura, pesticidas têm sido elaborados e utilizados com o objetivo de proteger as lavouras de pragas (ALI et al., 2020). Mesmo que os pesticidas aumentem o rendimento da safra, simultaneamente representam uma grave ameaça à estabilidade dos ecossistemas (ALI et al., 2020). O Brasil é um dos quatro principais países consumidores de agrotóxicos do mundo (PAUMGARTTEN, 2020), sendo o 2,4-D, a atrazina e o glifosato, os três pesticidas mais utilizados (BROVINI et al., 2021).

No estado de Goiás, um dos mais importantes celeiros agrícolas brasileiro, problemas relacionados à liberação de agrotóxicos tem sido reportados com frequência. Na mesorregião sul do estado, análise de amostras de águas superficiais (BORGES et al., 2019) e subterrâneas (ROCHA et al., 2015) tem indicado a presença de atrazina, resíduos oriundos de lavouras na região. Descartes incorretos de sementes de soja tratadas com agrotóxicos também é um problema negligenciado com capacidade de contaminar o solo e a água (OLIVEIRA JÚNIOR et al., 2022). Entre 2005 e 2015, houve mais de 2980 intoxicações humanas oriundas de pesticidas no estado (NEVES et al., 2020).

No entanto, é reconhecido que muitas formulações de agrotóxicos diminuem à medida que se decompõe ao longo do tempo; reportando níveis de resíduos mais elevados imediatamente após a aplicação, com diminuição gradual à medida que as safras vão crescendo (RIYAZ et al., 2021). Assim, os principais riscos dos agrotóxicos para organismos não alvo ocorrem no momento de sua aplicação e com isso, estudos avaliando o efeito em organismos

não alvo são importantes neste momento. Por outro lado, a maioria dos pesticidas como os organoclorados (Dieldrin, Lindano, Diclorodifeniltricloroetano e Diclorodifenildicloroetileno) são significativamente resistentes à biodegradação e, portanto, têm alto risco de entrar na cadeia alimentar, causando impacto adverso em espécies não-alvo, como, insetos polinizadores, pássaros, peixes, microrganismos benéficos e mamíferos (BAYAT et al., 2014; SAHA et al., 2020).

Os resíduos de pesticidas, se presentes no ar, no solo e na água, podem representar uma séria ameaça à diversidade biológica e à saúde humana (RIYAZ et al., 2021). Os corpos de água doce estão particularmente expostos ao risco de poluição por pesticidas devido sua penetração por meio do escoamento, deriva, lixiviação e drenagem (ALI et al., 2020). Estima-se que a perda de agrotóxicos do campo para o corpo d'água esteja entre 1 e 10% do total aplicado (ACAYABA et al., 2021), o que pode gerar implicações para a vida aquática e aqueles que dependem desses recursos hídricos.

Atrelados aos pesticidas, outros estressores químicos como os metais estão cada vez mais disseminados nos ecossistemas devido às atividades humanas (BEAUMELLE et al., 2021). O Brasil é um dos maiores exploradores de mineração do mundo (GIROTTTO et al., 2020), com papel marcante no desenvolvimento econômico e social do país (ALVES et al., 2021). Entretanto, essas atividades podem levar a contaminação ambiental como ocorreu em Mariana e Brumadinho, no estado de Minas Gerais.

Em Novembro de 2015 em Mariana, milhões de metros cúbicos (m^3) de rejeitos contendo principalmente argila, silte e metais pesados como ferro, cobre e manganês foram derramados (GOMES et al., 2018) contaminando ambientes terrestres e aquáticos. Anos depois, em Janeiro de 2019, outra mineradora colapsou liberando milhões de m^3 de rejeitos em Brumadinho, também em Minas Gerais (THOMPSON et al., 2020). Diante desses casos, compreender o impacto dos metais advindos da mineração na fauna selvagem e saúde humana é importante para gerar indicares de prevenção e sustentabilidade para a indústria da mineração.

Com base nesses estressores ambientais, como os pesticidas e metais pesados, os quirópteros têm sido considerados excelentes biodindicadores no Brasil e no mundo (DE SOUZA et al., 2020). Os morcegos pertencem a um grupo ecológico e taxonomicamente diverso, responsável por cerca de um quinto da diversidade dos mamíferos em todo o mundo (FRICK et al., 2020). Esses mamíferos possuem algumas particularidades, como longevidade notável, apesar de sua alta taxa metabólica, vivendo em média três vezes mais do que outros mamíferos de tamanho semelhante (LAGUNAS-RANGEL et al., 2020).

Atualmente são listadas 1447 espécies de morcegos no planeta distribuídos em 21 famílias e 237 gêneros (MDD 2023), cujo um total de 181 são descritos no Brasil (GARBINO et al., 2020). Eles estão expostos a inúmeras ameaças, incluindo poluição e doenças emergentes (CABLE et al., 2021), sendo os fatores antropogênicos possivelmente uma das forças motrizes para a diminuição constante das espécies (KUZUKIRAN et al., 2021). Isso ocorre, uma vez que costumam coexistir com humanos em áreas urbanas, industriais e agrícolas e tornam potencialmente expostos a uma variedade de poluentes ambientais (KUZUKIRAN et al., 2021). Eles podem ter contato com as substâncias químicas por meio de alimentos, água, contato dérmico ou inalação. Os morcegos bebem em bacias de rejeitos, que podem conter alto teor de cianeto, metais pesados e, ocasionalmente, pesticidas, dependendo da localização da operação de mineração (KORINE et al., 2016; HOOPER; AMELON 2022).

Nesse contexto, esse estudo avaliou a sensibilidade desses animais a poluentes ambientais no Brasil. Para isso, a literatura foi revisada. Concomitantemente, foram avaliados danos genotóxicos e mutagênicos em morcegos amostrados em áreas de soja, cana-de-açúcar e mineração no estado de Goiás, Brasil Central. Como biomarcador genotóxico, o ensaio cometa foi utilizado. Essa técnica é simples para medições de baixos níveis de danos e reparo de DNA em células individuais (MØLLER, 2022). Esse ensaio tem sido aplicado em diferentes organismos (invertebrados e vertebrados) em condições de estresse ambiental (GAJSKI et al., 2019a,b).

Já para análise mutagênica, o teste de micronúcleo em células esfoliadas de mucosa bucal foi aplicado. O aumento do número de micronúcleos em células é aceito como um indicador indireto das irregularidades cromossômicas numéricas e estruturais geradas por agentes ambientais nas células (YUKSEL; ARSAL YILDIRIM 2021). Esses dois métodos são minimamente invasivos, por não requererem a morte dos animais e por isso são utilizados em abordagens ecotoxicológicas.

Vários estudos têm alertado sobre áreas agrícolas com potencial a causar danos genotóxico e mutagênico em organismos não alvos no estado de Goiás (GONÇALVES et al., 2017; BORGES et al., 2019; SILVEIRA et al., 2021; BENVINDO-SOUZA et al., 2022), incluindo trabalhadores agrícolas (FRANCO et al., 2016; RAMOS et al., 2021). Nessa unidade federativa, a mineração de Níquel (Barro Alto e Niquelândia) também é considerada uma das principais áreas de extração do País e, até o momento, era incipiente os estudos que avaliaram a saúde de comunidade de morcegos nessa região. Para essa respectiva região de estudo, em algumas áreas dentro e próximas à fundição, as taxas de ingestão de poeira podem ser superiores a 50 mg/dia, o que pode acarretar riscos à saúde humana, caso não tenham medidas

de segurança, como o uso de máscaras (ETTLER et al., 2018). No entanto, para a fauna, no caso dos morcegos, isso possivelmente é um risco para a inalação.

Assim, com o presente estudo é possível identificar e classificar riscos para a fauna de morcegos. O conhecimento dos possíveis riscos é crucial para garantir a sobrevivência das espécies de morcegos (KUZUKIRAN et al., 2021) e tomar medidas para mitigá-las (RUSSO et al., 2021), seja em áreas agrícolas ou mineração. Finalmente, além dessa abordagem ecotoxicológica, a presente tese reporta a diversidade de morcegos amostradas nas respectivas áreas de estudo.

2. Objetivo geral

Investigar os efeitos genotóxico emutagênico em morcegos de paisagens transformada pela agricultura e mineração no cerrado brasileiro.

Objetivos específicos

- a) Identificar quantas e quais espécies de morcegos foram investigadas quanto aos impactos do uso de agrotóxicos e traços de metais pesados no Brasil. Além disso, descrevemos os principais efeitos dos contaminantes encontrados em morcegos para direcionar futuros caminhos de pesquisa;
- b) Analisar se espécies de morcegos de área agrícola de cultivo anual (soja/milho) e semi-perene (cana-de-açúcar) respondem da mesma maneira à perturbação ambiental por meio de danos genotóxico e mutagênico [frequência de lesão no DNA e formação de micronúcleos e anormalidades nucleares (células binucleadas, células com broto nuclear, células picnose, células cariorrexe e cariólise)]. Além disso, avaliou-se a relação entre a frequência de danos genotóxicos com atributos biológicos como, guildas tróficas e índice de massa corporal de morcegos neotropicais;
- c) Analisar se morcegos coletados em área de mineração de Ferroníquel responde com maior frequência de danos genotóxicos e mutagênicos quando comparados a espécies obtidas em área de referência (longe de atividade de mineração). Em conjunto com essa investigação, o sexo dos animais foi analisado para a frequência de danos;
- d) Avaliar a composição de morcegos em remanescentes do cerrado em regiões de cultivo de soja, cana e área de referência (Parque Nacional das Emas). Dados em anexo.

3. Estrutura da tese

Além do resumo e introdução geral, essa tese está dividida em quatro capítulos. Para os três primeiros capítulos, dois foram publicados e um está em revisão. Enquanto o último ainda não foi submetido.

Capítulo 1. de Souza, M. B., de Souza Santos, L. R., Borges, R. E., Nunes, H. F., Vieira, T.

B., Pacheco, S. M., e Silva, D. D. M. (2020). **Current status of ecotoxicological studies of bats in Brazil.** Bulletin of Environmental Contamination and Toxicology, 104(4), 393-399.

Capítulo 2. Benvindo-Souza, M., Hosokawa, A. V., Dos Santos, C. G. A., de Assis, R. A., Pedroso, T. M. A., Borges, R. E., Pacheco SM., Santos LRS, e Silva, D. D. M. (2022). **Evaluation of genotoxicity in bat species found on agricultural landscapes of the Cerrado savanna, central Brazil.** Environmental Pollution, 293, 118579.

Capítulo 3. Genotoxic, mutagenic, and cytotoxic analysis in bats in mining area. Estudo submetido/em revisão na Environmental Science and Pollution Research.

Capítulo 4. Mamíferos voadores em regiões com degradação crônica do Cerrado (Em anexo I).

4. Referências

- ACAYABA, R. D. A., DE ALBUQUERQUE, A. F., RIBESSI, R. L., DE ARAGÃO UMBUZEIRO, G., MONTAGNER, C. C. Occurrence of pesticides in waters from the largest sugar cane plantation region in the world. **Environmental Science and Pollution Research**, v. 28, n. 8, p. 9824-9835, 2021. <https://doi.org/10.1007/s11356-020-11428-1>
- ALVES, W., FERREIRA, P., ARAÚJO, M. Challenges and pathways for Brazilian mining sustainability. **Resources Policy**, v. 74, n. 101648, 2021. <https://doi.org/10.1016/j.resourpol.2020.101648>
- ALI, S., WALI, A. F., YATOO, A. M., MAJID, S., RASOOL, S., KHAN, R., ALI, M.N., WANI, J.A., FAROOQ, S., RASOOL, S., WANI, H.A., REHMAN, M. U. Effect of pesticides on fish fauna: Threats, challenges, and possible remedies. In **Bioremediation and Biotechnology**. Springer, Cham. pp. 27-54, 2020.
- ASSIS, R.A., REZENDE, W.R., DOS SANTOS, C.G.A. BENVINDO-SOUZA, M., AMORIM, N.P.L., BORGES, R.E., FRANCO-BELUSSI, L., DE OLIVEIRA, C., DE SOUZA SANTOS, L.R. Habitat differences affect the nuclear morphology of the erythrocytes and the hepatic melanin in Leptodactylusfuscus (Anura) in the Brazilian Cerrado savanna. **Environmental Science and Pollution Research**, 24. 2021. <https://doi.org/10.1007/s11356-021-14974-4>
- BAYAT, S., GEISER, F., KRISTIANSEN, P., WILSON, S.C. Organic contaminants in bats: trends and new issues. **Environ. Int.** v. 63, p.40-52, 2014.
- BEAUMELLE, L., THOUVENOT, L., HINES, J., JOCHUM, M., EISENHAUER, N., & PHILLIPS, H.R. Soil fauna diversity and chemical stressors: a review of knowledge gaps and roadmap for future research. **Ecography**, v. 44, n. 6, p. 845-859. 2021.
- BENVINDO-SOUZA, M., HOSOKAWA, A. V., DOS SANTOS, C. G. A., DE ASSIS, R.A., PEDROSO, T.M.A., BORGES, R. E., PACHECO SM., SANTOS LRS, E SILVA, D.D. M. Evaluation of genotoxicity in bat species found on agricultural landscapes of the Cerrado savanna, central Brazil. **Environmental Pollution**, v.293, p. 118579, 2022.
- BROVINI, E. M., DE DEUS, B. C. T., VILAS-BOAS, J. A., QUADRA, G.R., CARVALHO, L., MENDONÇA, R. F., PEREIRA R.O., CARDOSO, S.J. Three-bestseller pesticides in Brazil: Freshwater concentrations and potential environmental risks. **Science of The Total Environment**, v. 771, p. 144754, 2021. <https://doi.org/10.1016/j.scitotenv.2020.144754>
- CABLE, A. B., WILLCOX, E. V., LEPPANEN, C. Contaminant exposure as an additional

- stressor to bats affected by white-nose syndrome: current evidence and knowledge gaps. **Ecotoxicology**, p. 1-12, 2021. <https://doi.org/10.1007/s10646-021-02475-6>
- BORGES, R. E., DE SOUZA SANTOS, L. R., BENVINDO-SOUZA, M., MODESTO, R. S., ASSIS, R. A., DE OLIVEIRA, C. Genotoxic evaluation in tadpoles associated with agriculture in the Central Cerrado, Brazil. **Archives of environmental Contamination and Toxicology**, v. 77, n.1, p. 22-28, 2019.
- BRAZEIRO, A., ACHKAR, M., TORANZA, C., BARTESAGHI, L. Agricultural expansion in Uruguayan grasslands and priority areas for vertebrate and woody plant conservation. **Ecologyand Society**, v.25, n.1, 2020.
- DE SOUZA, M. B., DE SOUZA SANTOS, L. R., BORGES, R. E., NUNES, H. F., VIEIRA, T. B., PACHECO, S. M., E SILVA, D. D. M. Current status of ecotoxicological studies of bats in Brazil. **Bulletin of Environmental Contamination and Toxicology**, v. 104, n. 4, p. 393-399, 2020.
- ETTLER, V., POLÁK, L., MIHALJEVIČ, M., RATIÉ, G., GARNIER, J., & QUANTIN, C. Oral bioaccessibility of inorganic contaminants in waste dusts generated by laterite Ni ore smelting. **Environmental geochemistryandhealth**, v. 40, n. 5, 1699-1712, 2018.
- FRANCO, F. C., ALVES, A. A., GODOY, F. R., AVELAR, J. B., RODRIGUES, D. D., PEDROSO, T. M. A., CRUZ AD, NOMURA F, E SILVA, D. D. M. Evaluating genotoxic risks in Brazilian public health agents occupationally exposed to pesticides: a multi-biomarker approach. **Environmental Science and Pollution Research**, v. 23, n. 19, p. 19723-19734, 2016.
- FRICK, W. F., KINGSTON, T., FLANDERS, J. A review of the major threats and challenges to global bat conservation. **Annals of the New York Academy of Sciences**, v. 1469, n. 1, 5-25, 2020.
- GAJSKI, G, ZEGURA, B, LADEIRA, C, POURRUT, B, DEL BO', C, NOVAK, M, SRAMKOVA, M, MILIC, M, GUTZKOW, KB, COSTA, S, DUSINSKA, M, BRUNBORG, G, COLLINS, A. The comet assay in animal models: From bugs to whales - (Part 1 Invertebrates). **Mutation Research-Reviews in Mutation Research**, v. 779, p. 82-113, 2019a. <https://doi.org/10.1016/j.mrrev.2019.02.003>
- GAJSKI, G, ZEGURA, B, LADEIRA, C, NOVAK, M, SRARNKOVA, M, POURRUT, B, DEL BO', C, MILIC, M, GUTZKOW, KB, COSTA, S, DUSINSKA, M, BRUNBORG, G, COLLINS, A. The comet assay in animal models: From bugs to whales - (Part 2 Vertebrates). **Mutation Research-Reviews in Mutation Research**, v. 781, p. 130-164, 2019b.

GARBINO, G.S.T., R. GREGORIN, I.P. LIMA, L. LOUREIRO, L.M. MORAS, R. MORATELLI, M.R. NOGUEIRA, A.C. PAVAN, V.C. TAVARES, M.C. DO NASCIMENTO AND A.L. PERACCHI. 2020. Updated checklist of Brazilian bats: versão 2020. Comitê da Lista de Morcegos do Brasil—CLMB. **Sociedade Brasileira para o Estudo de Quirópteros** (Sbeq).<<https://www.sbeq.net/lista-de-especies>> acessado em: 06 de Janeiro de 2022

GIROTTA, L., ESPÍNDOLA, E. L. G., GEBARA, R. C., FREITAS, J. S. Acute and chronic effects on tadpoles (*Lithobatescatesbeianus*) exposed to mining tailings from the dam rupture in Mariana, MG (Brazil). **Water, Air, & Soil Pollution**, v. 231, n. 7, p. 1-15, 2020.

GOMES, L. C., CHIPPARI-GOMES, A. R., MIRANDA, T. O., PEREIRA, T. M., MERÇON, J., DAVEL, V. C., ... RAMOS, J. P. L. Genotoxicity effects on *Geophagusbrasiliensis* fish exposed to Doce River water after the environmental disaster in the city of Mariana, MG, Brazil. **Brazilian Journal of Biology**, v. 79, n. 659-664, 2018.

GONÇALVES, M.W., GAMBALE, P.G., GODOY, F.R., ALVES, A.A., REZENDE, P.H., CRUZ, A. D. D., MACIEL NM, NOMURA F., BASTOS RP., MARCO-JR, P., SILVA, D. D.M. The agricultural impact of pesticides on *Physalaemus cuvieri* tadpoles (Amphibia: Anura) ascertained by comet assay. **Zoologia (Curitiba)**, v. 34, 2017.

HOOPER, S., & AMELON, S. **Pesticide Exposure Risks to Chiropteran Species and the Impacts on Emerging Zoonotic Diseases**, 2022.

KORINE C, ADAMS R, RUSSO D, FISHER-PHELPS M, JACOBS D. Bats and water: Anthropogenic alterations threaten global bat populations. In: Voigt CC, Kingston T, editors. *Bats in the Anthropocene: Conservation of Bats in a Changing World*. Cham: Springer International Publishing. p. 215-241, 2016. https://doi.org/10.1007/978-3-319-25220-9_8

KUIPERS, K. J., HILBERS, J. P., GARCIA-ULLOA, J., GRAAE, B. J., MAY, R., VERONES, F., HUIJBREGTS M.A.J., SCHIPPER, A. M. Habitat fragmentation amplifies threats from habitat loss to mammal diversity across the world's terrestrial ecoregions. **One Earth**, v. 4, n. 10, p. 1505-1513, 2021.

KUZUKIRAN, O., SIMSEK, I., YORULMAZ, T., YURDAKOK-DIKMEN, B., OZKAN, O., FILAZI, A. Multi Residues of Environmental Contaminants in Bats from Turkey. **Chemosphere**, n. 131022, 2021.

LAGUNAS-RANGEL, F. A. Why do bats live so long?—Possible molecular mechanisms. **Biogerontology**, v. 21, n. 1, p. 1-11, 2020. <https://doi.org/10.1007/s10522-019-09840-3>

MØLLER, P. Measurement of oxidatively damaged DNA in mammalian cells using the comet assay: Reflections on validity, reliability and variability. **Mutation Research/Genetic Toxicology and Environmental Mutagenesis**, v. 873, n. 503423, 2022. <https://doi.org/10.1016/j.mrgentox.2021.503423>

MDD (2023) **Mammal Diversity Database. Explore Current Mammalian Taxonomy.** Disponível em: <https://www.mammaldiversity.org/taxa.html>. Acesso em 06 de Janeiro de 2022.

NEVES, P. D. M., MENDONÇA, M. R., BELLINI, M., PÔSSAS, I. B. Poisoning by agricultural pesticides in the state of Goiás, Brazil, 2005-2015: analysis of records in official information systems. **Ciência & Saúde Coletiva**, 25, 2743-2754, 2020. <https://doi.org/10.1590/1413-81232020257.09562018>

OLIVEIRA JÚNIOR, C. I., CARDOSO, A. T., GOULART, A. C., OLIVEIRA, M. A., SANTOS, J. P. V., & GOULART, S. M. Determination of Pesticides in Soybean Seeds Incorrectly Discarded Near a Spring of the Paranaíba River, GO-Brazil. **Chemistry & Biodiversity**, 19(1), e202100560, 2022. <https://doi.org/10.1002/cbdv.202100560>

PAUMGARTTEN, F. J. Pesticides and public health in Brazil. **Current Opinion in Toxicology**, v. 22, p. 7-11, 2020. <https://doi.org/10.1016/j.cotox.2020.01.003>

RAMOS, J.S.A., PEDROSO, T.M.A., GODOY, F.R., BATISTA, R.E., DE ALMEIDA, F.B., FRANCELIN, C., RIBEIRO FL., PARISE MR., SILVA, D.D.M. Multi-biomarker responses to pesticides in an agricultural population from Central Brazil. **Science of the Total Environment**, v. 754, n. 141893, 2021.

RIYAZ, M., SHAH, R. A., SIVASANKARAN, K. Pesticide Residues: Impacts on Fauna and the Environment. 2021. <https://doi.org/10.5772/intechopen.98379>

ROCHA, A. A., MONTEIRO, S. H., ANDRADE, G. C., VILCA, F. Z., TORNISIELO, V. L. Monitoring of pesticide residues in surface and subsurface waters, sediments, and fish in center-pivot irrigation areas. **Journal of the Brazilian Chemical Society**, 26, 2269-2278, 2015. <https://doi.org/10.5935/0103-5053.20150215>

RUSSO, D., SALINAS-RAMOS, V.B., CISTRONE, L., SMERALDO, S., BOSSO, L., ANCILLOTTO, L. Do We Need to Use Bats as Bioindicators?. **Biology**, v. 10, n. 8, p. 693, 2021. <https://doi.org/10.3390/biology10080693>

SAHA LA., KISHOR V., BAUDDH K. Impacts of Synthetic Pesticides on Soil Health and Non-targeted Flora and Fauna. In: Bauddh K., Kumar S., Singh R., Korstad J. (eds) **Ecological and Practical Applications for Sustainable Agriculture**. Springer, Singapore, 2020. https://doi.org/10.1007/978-981-15-3372-3_4

SILVEIRA, E.D.R., BENVINDO-SOUZA, M., ASSIS, R.A., DOS SANTOS, C.G.A., DE LIMA AMORIM, N.P., BORGES, R. E., MELO, C., DE SOUZA SANTOS, L.R. Micronucleus and different nuclear abnormalities in wild birds in the Cerrado, Brazil. **Environmental Science and Pollution Research**, 2021.
<https://doi.org/10.1007/s11356-021-16845-4>

THOMPSON, F., DE OLIVEIRA, B. C., CORDEIRO, M. C., MASI, B. P., RANGEL, T. P., PAZ, P., ... DE REZENDE, C. E. Severe impacts of the Brumadinho dam failure (Minas Gerais, Brazil) on the water quality of the Paraopeba River. **Science of the Total Environment**, v. 705, p. 135914, 2020.

YUKSEL, B.; ARSAL YILDIRIM, S. Determination of cytogenetic abnormalities in buccal mucosa of dental laboratory technicians. **Archives of Environmental & Occupational Health**, p. 1-8, 2021. <https://doi.org/10.1080/19338244.2021.1943641>

CAPÍTULO 1

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PERSPECTIVE



Current Status of Ecotoxicological Studies of Bats in Brazil

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Abstract

Bats are sensitive to contaminants generated by agricultural activities, mining, and urbanization. In this review, we update the status of bat toxicology in Brazil. Agriculture, for example, in addition to habitat fragmentation and loss, undoubtedly affects non-target organisms through the use of pesticides. Other factors such as trace metals are a neglected problem in the country, as they can deposit on insects and plants reaching bats through the ingestion of these foods. Of the 181 species of bats in the country, only 4.9% have been investigated. The frugivorous species, *Artibeus lituratus*, has frequently been studied for the effects of pesticide exposure, and impacts at the cellular level on metabolism and reproduction have been observed. Given the scarcity of studies on bat ecotoxicology, we encourage national researchers and scientists elsewhere to increase knowledge of the effects of chemical contaminants on bats in Brazil.

Keywords: Bats; Contaminants; Pesticides; Metals; Bioaccumulation; Bioindicator species

Introduction

Bats are among the most abundant mammals, with colonies reaching in the millions, and represent some of the largest concentrations of organisms on Earth (Hammerson et al. 2017). In Brazil, which has one of the most considerable diversity of bats in the world (Nunes et al. 2017), 181 species are recognized, distributed in 72 genera and nine families (Dias et al. 2016). Among the ecological roles played by bats, seed dispersal and pollination, contributing to the reforestation of degraded areas and maintenance of plant genetic diversity and the control of insect populations, including agricultural and urban pests. However, several factors

may influence the distribution of their diversity among environments. The development of wind power as a source of energy (Hammerson et al. 2017), habitat fragmentation and loss, climate change, air pollution and diseases such as white-nose syndrome are some of the leading causes of population decline in many bat species around the world (Rodhouse et al. 2012).

The diversified bat diet, which enables the various environmental services, is also a vulnerability, as it causes the group to be subject to different sources of contaminants (Jones et al. 2009). In general, species that forage in agricultural areas are exposed to pesticides by ingesting fruits (Oliveira et al. 2017) or contaminated insects (Bayat et al. 2014; Stahlschmidt et al. 2017). Similarly, trace heavy metal can bioaccumulate in plants and insects and pass through the trophic chain reaching bats (Zukal et al. 2015). Since environmental contamination is a topic of great interest which directly affects the quality of ecosystems and all living organisms (Ferrante et al. 2018b), the impact of chemical contaminants on bats is well-documented, however, for tropical regions like Brazil, toxicological research is still incipient. Thus, our main objective was to identify how many and which bat species are investigated for the impacts of pesticide use and trace heavy metal in Brazil. In addition, we describe the main effects of contaminants found in bats to direct future research pathways.

Literature Screening

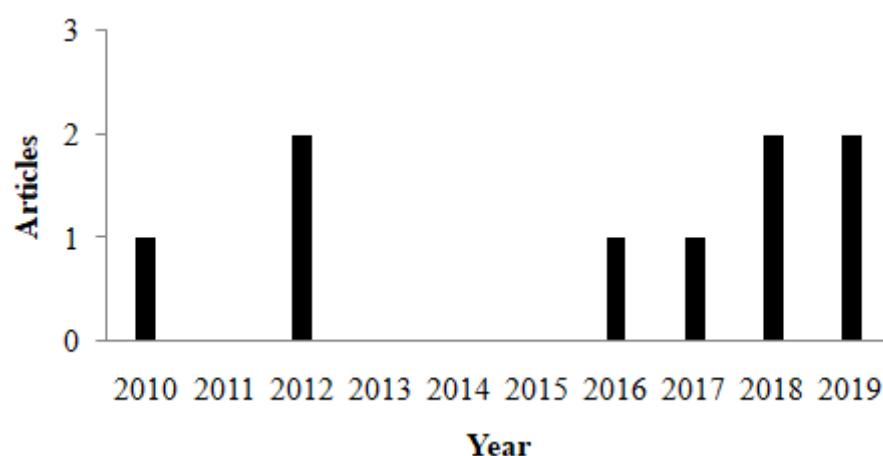
Scientific research on bat toxicology in Brazil was investigated using four databases, Web of Science (www.isiknowledge.com), Scopus (www.scopus.com), Scielo (www.scielo.org) and Google Scholar (<https://scholar.google.com>). The keywords used were: bat* combined with variables, pesticides, heavy metals, and pollution. The same combinations in Portuguese were used in order to broaden the search of public works, whose objective was to test for exposure of these contaminants (pesticides, heavy metals, or other environmental pollution) and subsequent bioaccumulation and physiological or cellular damage. Theses, dissertations, and abstracts were not incorporated into the database in order to avoid overestimating/overlapping search results, as they tend to be published in the form of papers. Database searches took place up to September 2019.

The scenario of bat ecotoxicology in Brazil

Our national review reports nine studies from 2010 to September 2019 (Fig 1). Nine bat species distributed among insectivores, frugivores, and nectarivores were the subjects of toxicological investigations (Table 1), which is equivalent to 4.9% of Brazilian bat species.

One possible reason for this low number of studies may be the recent emergence of ecotoxicology in bats in Brazil, as in other tropical countries (Sueitt et al. 2015; Duarte et al. 2017). In contrast, temperate regions have investigated bats more often (Zukal et al. 2015). The United States, for example, has approximately 45 bat species (Hammerson et al. 2017) when compared to 181 species in Brazil; however, they are pioneers in the ecotoxicology of this taxa for contaminant-related issues (Bayat et al. 2014; Zukal et al. 2015). Thus, based on species diversity in Brazil, the country has great need and future potential for investigations and related to the impacts of chemical contaminants on chiroptero fauna.

Fig. 1 Temporal trend in articles publications on the impact of contaminants on bats in Brazil.



The first study in Brazil was published in 2010, investigating the metal content (chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), aluminum (Al) and silicon (Si)) in animals obtained from the Santa Catarina coal basin (Zocche et al. 2010). The analysis quantified bioaccumulation of Cr, Ni, Cu, and Pb in *Molossus molossus* liver, and Cu and Fe in *Tadarida brasiliensis* in higher concentrations when compared to a reference area away from mining activities. In addition, *T. brasiliensis* from the mining area showed more significant DNA damage, according to the comet assay parameters (Zocche et al. 2010). These results corroborate the fact that toxic metals such as these bioaccumulate in insectivorous mammals (Walker et al. 2007). Moreover, in this Brazilian study, six metals (Cr, Mn, Ni, Cu, Cd and Pb) were observed, which in turn are part of those eleven cited by Zukal et al. (2015) as the most concerning for wildlife.

In general, some metals are essential for life, while others are considered chemically toxic because they have no known biological function in the body (Ferrante et al. 2018b). Metals such as Cu, Fe, and Zn, although considered essential, persist in their circulatory systems and are capable of crossing the blood-brain barrier of bats, which can have adverse effects on critical functions such as flight and echolocation (Hill et al. 2018). On the other hand, the metal Cd can be progressively bioaccumulated over time (Walker et al. 2002), which

is concerning since some species of bats have long life spans. Streit and Nagel (1993) observed that bats received maternal heavy metals through lactation, that Cu and Cr are effectively transferred this way, and that Pb and Cd were released only moderately from maternal tissue. Researchers have also noted similar transfers with pesticides. Male bats may show higher concentrations of contaminants because females pass residues through milk during lactation (Bayat et al. 2014).

In the following years, the Brazilian scientific trend mainly was focused on agricultural pesticides (Table 1). Results from these studies, such as the investigation of organophosphorous insecticides such as fenthion, demonstrated metabolic and histopathological changes in *Artibeus lituratus* (Amaral et al. 2012a). Specifically, fenthion affected some components of energy metabolism (increase of muscle glycogen and decrease of carcass fatty acids) and promoted changes in the morphology (increase in diameter) of hepatocytes. Amaral et al. (2012a) underlined that pathological evidence in hepatocytes, coupled with a decrease in carcass fatty acid content may impair bat adaptations to changes in the environment, including seasonal events and reproduction, which may affect bat population dynamics.

Another study with the insecticide spinosyn, Amaral et al. (2012b) emphasized that its use at the recommended label rate did not affect the general energy metabolism in *A. lituratus*, but may compromise some ultrastructural characteristics of hepatocytes. On the other hand, chronic exposure to low concentrations of endosulfan induced bioaccumulation and decreased fatty acid content in *A. lituratus* species, indicating a substantial impairment to the health of this critical seed disperser in neotropical forests (Brinati et al. 2016). Oliveira et al. (2017) also evaluated low doses of endosulfan concentrations (0,1.05 and 2.1 g/l) and highlighted that this insecticide might induce sublethal effects such as liver morphological changes, including cell degeneration and death, with apparent lipid accumulation in cytoplasm (steatosis) and pyknotic nuclei, karyolysis and deposition of collagen fibers.

Regarding exposure to pyrethroids such as deltamethrin, doses of 0.02 and 0.04 mg/kg on papaya (offered to frugivorous bats) resulted in an increase in the enzymes aspartate aminotransferase and alanine aminotransferase, and also hyperglycemia. In addition to this, liver and muscle pectoral disease presenting oxidative stress was seen, which has direct consequences on flight capacity, reproduction and metabolism (Oliveira et al. 2018). Tests also performed with the fungicide tebuconazole (1 ml/L) on the testicular, and epididymal histomorphometry of *A. lituratus* showed testicular and epididymal morphometric alterations, especially at 30-day exposure, suggesting that functional alterations may be occurring in these

organs and negatively influencing their capacity (Machado-Neves et al. 2018).

Exposure to contaminants, particularly pesticides, has been implicated as an important threat factor contributing to decreases in bat populations worldwide (Bayat et al. 2014). The risks associated with exposure to persistent compounds such as some pesticides have similar potential when compared to certain heavy metals, especially in the aspect of transfer along the food chain and bioaccumulation. The effects of agricultural contaminants on bats, however, are still poorly understood, particularly sublethal effects, due to difficulties in sampling populations, monitoring exposures, and relating exposure to effects (Bayat et al. 2014). In this context, we saw that despite the few experimental studies with *A. lituratus* in Brazil, a wide range of negative impacts were observed. These impacts mainly related to energy metabolism and testicular damage, and undoubtedly are one of the main bases for urgent attention to address indiscriminate pesticide use, as bats have low reproductive rates (Ferrante et al. 2018a), and these impacts may culminate in rapid species declines.

More recently, in order to broaden the understanding of the effects of environmental contaminants on bats, we have adapted the micronucleus test for exfoliated oral mucosa cells (Benvindo-Souza et al. 2019a,b). The test showed suitable parameters and accuracy in the score of nuclear buds, binucleated cells, condensed chromatin, karyorrhexis, pyknosis, and karyolysis. It was able to identify genetic damage in bats harvested from soybean areas when compared to urban environments, also identifying which insectivorous bats are the most susceptible, followed by frugivores and nectarivores (Benvindo-Souza et al. 2019b). In this context, these ecotoxicological data were obtained by a series of techniques, the main ones being gas chromatography, histopathological biomarkers, comet assay, and micronucleus test (Fig 2).

Table 1. The Brazilian contribution to scientific publications on contaminants in bats.

Species	Trophicguild	Source of contamination		Samples	References
		Pesticides	Metals		
<i>Molossus smolossus</i> ,	Insectivore	-	Al, Si, Cr,	Blood and liver	Zocche et al. 2010
<i>Tadarida brasiliensis</i> ,			Mn, Fe, Ni,		
<i>Eptesicus diminutus</i>			Cu, Zn, Cd, Pb		
	Fenthion	-		Blood, liver, hind limb, forelimb, breast muscles, and adipose tissue	Amaral et al. 2012a

	Spinosyn	-	Liver cells, forelimb muscles, hind limb	Amaral et al. 2012b
Frugivore			muscles and breast muscle	
	Endosulfan	-	Liver, hind limb, forelimb and breast	Brinati et al. 2016
			muscles and adipose tissue	
<i>Artibeus lituratus</i>	Endosulfan	-	Liver and kidneys	Oliveira et al. 2017
	Deltamethrin		Blood, liver and pectoral muscle	Oliveira et al. 2018
	Tebuconazole		Testis and epididymis	Machado- Neves et al. 2018
<i>Nyctinomops</i> <i>laticaudatus</i> , <i>Noctilio</i> <i>albiventris</i> , <i>Pteronotus</i> <i>parnellii</i>	Insectivore	-	Exfoliated cells of buccal mucosa	Benvindo- Souza et al. 2019a
<i>Artibeus lituratus</i> ,	Frugivore,	-	Exfoliated cells of	Benvindo-
<i>Artibeus planirostris</i> ,	Insectivore		buccal mucosa	Souza et al.
<i>Molossus molossus</i> ,	Nectarivore			2019b
<i>Glossophaga soricina</i> ,				
<i>Nyctinomops</i> <i>laticaudatus</i>				

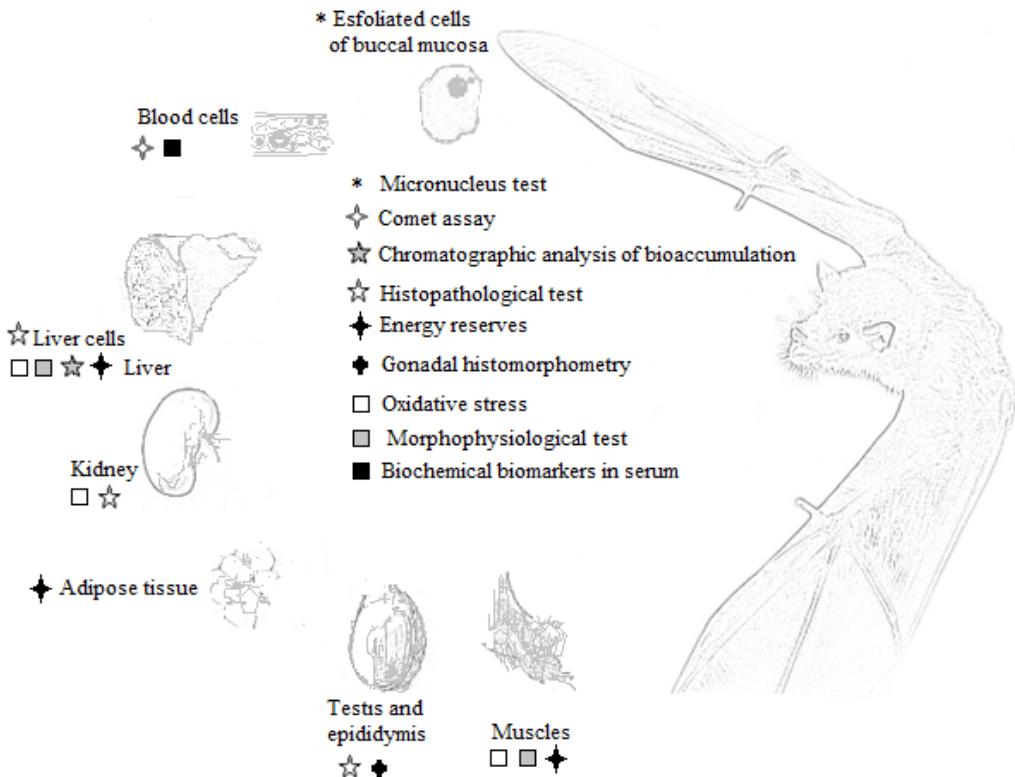


Fig. 2 Main tissues, distinct biomarkers and methodologies evaluated for bat ecotoxicological research in Brazil.

Future perspectives

Overall, the number of publications on bat ecotoxicology in Brazil is still rather small and most are very recent. Raising interest in the creation of new research groups on this topic in Brazil is still a challenge, although it is necessary, especially when considering the accelerated anthropization of natural ecosystems. There is great concern that easing environmental conservation laws in the country to boost the agribusiness sector will have substantial impacts on biodiversity. Recent events involving industrial disasters, such as the breach of the Fundão dam in Bento Rodrigues, Mariana, and the Brumadinho stream, both in the state of Minas Gerais, have raised concern among both the academic community and society in general, on the effects of contaminants such as heavy metals on biodiversity.

Data on trace heavy metals in taxa such as bats are still incipient, although these residues contaminate a wide range of habitats (Hernout et al. 2016). Other contaminants such as pesticides have elevated Brazil to the ranking of the world's largest consumers of pesticides (Bernieri et al. 2019), and some *in situ* studies have shown the impact of these compounds on different taxonomic groups such as anurans (Gonçalves et al. 2017; Borges et al. 2019), fishes (Vieira et al. 2016), birds (Souto et al. 2018) and humans (Bernieri et al. 2019; Godoy et al.

2019). Considering bats as organisms with potential plasticity, flight ability, and diverse feeding habits, ecotoxicological studies favor the understanding of the impact of contaminants on biodiversity and a warning for the integrity of human well-being.

Several other review studies have warned of the sensitivity of bats to chemical contaminants (Bayat et al. 2014; Russo and Ancillotto 2015; Zukal et al. 2015; Salvarina 2016), which makes tracing exposure difficult as these animals are present in urban, industrial and agricultural landscapes. Studies conducted in Brazil's new agricultural frontier, the Matopiba region, a continuous zone formed by the states of Maranhão, Tocantins, Piauí, and Bahia, located mainly within the Cerrado biome (Araujo et al. 2019) have demonstrated nuclear alterations of exfoliated mucosal cells of insectivorous bats as well as frugivorous bats from the municipality of Palmas, Tocantins (Benvindo-Souza et al. 2019*a,b*). However, further studies are needed to elucidate the relationship between cell damage and pesticide exposure. Understanding this mechanism is relatively important as the route of contaminant exposure occurs through ingestion, dermally, and through inhalation (Ferrante et al. 2018*a*). Agents such as dust or chemical contaminants present in the urban troposphere, for example, can impair bat health and survival (Voigt et al. 2018). Hariano et al. (1993) observed that high lead concentrations in frugivorous bat hair correlated closely with kidney and liver concentrations, reflecting exposure likely resulting from urban atmospheric sources.

Finally, heavy metals from mining, urban contaminants or pesticides are just one of the threats faced by bats in Brazil. Natural bat populations end up being hampered by fragmentation and habitat loss, in particular by converting land to livestock grazing and agriculture, converting cave areas into mining sites, or urbanization itself (Melo et al. 2012; Farneda et al. 2015; Russo and Ancillotto 2015). Low cost techniques such as genotoxic and mutagenic analysis (comet assay and micronucleus test) are recommended for the evaluation of bat sensitivity in natural environments (Zocche et al. 2010; Benvindo et al. 2019*a,b*). Parameters such as age, gender, and seasonality are variables that can be investigated in future work. In addition to using bat tissues (Valdespino and Sosa 2017), we encourage analytical techniques using hair (Flache et al. 2015), guano (Zukal et al. 2015) or other non-lethally obtained samples as tools for monitoring exposure to different levels of trace metals.

As far as hair, it is recognized that the hair root continually is in contact with the bloodstream and therefore may incorporate metals that circulate in the blood during growth (Mina et al. 2019), in addition to storing external airborne particles. Concomitantly, the wing membrane is also likely to accumulate some metals, so they are indicated for ecotoxicological analysis in bats (Mina et al. 2019). Thus, we draw attention to the importance of not only

ecological research but also of ecotoxicological research. It is important to better understand exposure, effects, and mechanisms involved in the bioaccumulation of heavy metals and pesticides in bats, especially as it relates to protection of bats and the critical ecosystem services they provide, for example, insectivorous bats feed on insect pests, thus reducing the need for pesticides (López-Hoffman et al. 2019). Thus, such data will be useful to indicate areas of risk and generate scientific subsidies for environmental management strategies, as well as projections for food safety of Brazilian products, such as agricultural crops.

Final considerations

Herein, we review the recent status of bat ecotoxicology in Brazil. Contaminants such as pesticides have been the focus of national investigations. The reason for this scientific trend is linked to the agricultural advances in the country. Although biomarkers of physiological interest have been the most used in ecotoxicological diagnostics, we also encourage the use of genotoxicity and mutagenicity tests in bat communities. We emphasize obtaining a greater *in situ* sampling effort to detect bat sentinel species. Among the significant impacts, even those related to energy reserve, reproductive system, genotoxic and mutagenic effects, when acting alone or synergistically, could significantly compromise the survival of wild animals such as bats. Thus, given the lack of studies, we invite national and international researchers around the world to join us in interest in this topic in Brazil.

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References

- Amaral TS, Carvalho TF, Silva MC, Goulart LS, Barros MS, Picanco MC, Neves CA, Freitas MB (2012a) Metabolic and histopathological alterations in the fruit-eating bat *Artibeus lituratus* induced by the organophosphorous pesticide fenthion. Acta Chiropterologica 14:225-232
- Amaral TS, Carvalho TF, Silva MC, Barros MS, Picanco MC, Neves CA, Freitas MB (2012b) Short-term effects of a spinosyn's family insecticide on energy metabolism and liver morphology in frugivorous bats *Artibeus lituratus* (Olfers, 1818). Braz J Biol 72:299-304
- Araujo MLS, Sano EE, Bolfe EL, Santos JRN, dos Santos JS, Silva FB (2019) Spatio temporal

- dynamics of soybean crop inthe Matopiba region, Brazil (1990-2015). Land Use Policy 80:57-67
- Bayat S, Geiser F, Kristiansen P, Wilson SC (2014) Organic contaminants in bats: trends and new issues. Environ Int 63:40-52
- Benvindo-Souza M, Borges RE, Pacheco SM, Santos LRS (2019a) Micronucleus and other nuclear abnormalities in exfoliated cells of buccal mucosa of bats at different trophic levels. Ecotoxicol Environ Saf 172:120-127
- Benvindo-Souza M, Borges RE, Pacheco SM, Santos LRD (2019b) Genotoxicological analyses of insectivorous bats (Mammalia: Chiroptera) in central Brazil: the oral epithelium as an indicator of environmental quality. Environ Pollut 245:504-509
- Bernieri T, Rodrigues D, Randon Barbosa I, Perassolo MS, GrolliArdenghi P, Basso da Silva L (2019) Effect of pesticide exposure on total antioxidant capacity and biochemical parameters in Brazilian soybean farmers. Drug Chem Toxicol 5:1-7 doi:10.1080/01480545.2019.1566353
- Borges RE, Santos LRS, Benvindo-Souza M, Modesto RS, Assis RA, Oliveira C (2019) Genotoxic evaluation in tadpoles associated with agriculture in the central Cerrado, Brazil. Arch Environ Contam Toxicol 77:22-28
- Brinati A, Oliveira JM, Oliveira VS, Barros MS, Carvalho BM, Oliveira LS, Queiroz MEL, Matta SLP, Freitas MB (2016) Low, chronic exposure to endosulfan induces bioaccumulation and decreased carcass total fatty acids in neotropical fruit bats. Bull Environ Contam Toxicol 97:626-631
- Díaz MM, Solari S, Aguirre LF, Aguiar LMS, Barquez RM (2016) Clave de identificación de losmurciélagos de Sudamérica. Tucumán, Programa de Conservación de losMurciélagos de Argentina
- Duarte LFD, Souza CA, Pereira CDS, Pinheiro MAA (2017) Metal toxicity assessment by sentinel species of mangroves: *in situ* case study integrating chemical and biomarker analyses. Ecotoxicol Environ Saf 145:367-376
- Farneda FZ, Rocha R, Lopez-Baucells A, Groenenberg M, SILVA I, Palmeirim JM, Bobrowiec PED, Meyer CFJ (2015) Trait-related responses to habitat fragmentation in Amazonian bats. J Appl Ecol 52:1381-1391
- Ferrante M, SpinaMT, Hernout BV, Grasso A, Messina A, Grasso R, Agnelli P, Bruno MV, Copat C (2018a) Trace elements bioaccumulation in liver and fur of *Myotismyotis* from two caves of the eastern side of Sicily (Italy): a comparison between a control and a polluted area. Environ Pollut 240:273-285

- Ferrante M, Signorelli SS, Ferlito SL, Grasso A, Dimartino A, Copat C (2018b) Groundwater-based water wells characterization from Guinea Bissau (western Africa): a risk evaluation for the local population. *Sci Total Environ* 619–620:916-926
- Flache L, Czarnecki S, Durante RA, Kierdorf U, Encarnacao ME (2015) Trace metal concentrations in hairs of three bat species from an urbanized area in Germany. *J Environ Sci* 31:184-193.
- Godoy FR, Nunes HF, Alves AA, Carvalho WF, Franco FC, Pereira RR, Cruz AS, da Silva CC, Bastos RP, Silva DM (2019) Increased DNA damage is not associated to polymorphisms in OGG1 DNA repair gene, CYP2E1 detoxification gene, and biochemical and hematological findings in soybeans farmers from Central Brazil. *Environ Sci Pollut Res Int* 26:26553-26562.
- Gonçalves MW, Gambale PG, Godoy FR, Alves AA, Rezende PHA, Cruz AD, Maciel NM, Nomura F, Bastos RP, Marco-Jr P, Silva DM (2017) The agricultural impact of pesticides on *Physalaemus cuvieri* tadpoles (Amphibia: Anura) ascertained by comet assay. *Zoologia* 34:e19865
- Hammerson GA, Kling M, Harkness M, Ormes M, Young BE(2017) Strong geographic and temporal patterns in conservation status of North American bats. *Biol Conserv* 212:144-152
- Hariono B, Ng J, Sutton RH (1993) Lead concentrations in tissues of fruit bats (*Pteropusspp*) in urban and nonurban locations. *Wildlife Research* 20:315-320
- Hernout BV, Arnold KE, McClean CJ, Walls M, Baxter M, Boxall ABA(2016) A national level assessment of metal contamination in bats. *Environ Pollut* 214:847-858
- Hill K, Schoeman MC, VoslooD(2018) The brains of bats foraging at wastewater treatment works accumulate arsenic, and have low non-enzymatic antioxidant capacities. *Neurotoxicology* 69:232-241
- Jones G, Jacobs DS, Kunz TH, Willig MR, Racey PA (2009) Carpe noctem: the importance of bats as bioindicators. *Endang Species Res* 8:93-115
- López-Hoffman G, Chester DC, Semmens DJ, Thogmartin WE, Rodriguez-McGoffin MS, Merideth R, Diffendorfer JE (2019) Ecosystem services from transborder migratory species: implications for conservation governance. *Ann Rev Environ Resour* 42:509-539
- Machado-Neves M, Neto MJO, Miranda DC, Souza ACF, Castro MM, Sertorio MN, Carvalho TF, Matta SLP, Freitas MB(2018) Dietary exposure to tebuconazole affects testicular and epididymal histomorphometry in frugivorous bats. *Bull Environ Contam Toxicol* 101:197-204

- Melo BES, Barros MS, Carvalho TF, Amaral TS, Freitas MB (2012) Energy reserves of *Artibeuslituratus* (Chiroptera: Phyllostomidae) in two areas with different degrees of conservation in Minas Gerais, Brazil. *Braz J Biol* 72:181-187
- Mina R, Alves J, da Silva AA, Natal-da-Luz T, Cabral JA, Barros P, Topping CJ, Sousa JP (2019) Wing membrane and fur samples as reliable biological matrices to measure bioaccumulation of metals and metalloids in bats. *Environ Pollut* 253:199-206.
- Nunes H, Rocha FL, Codeiro-Estrela P (2017) Bats in urban areas of Brazil: roosts, food resources and parasites in disturbed environments. *Urban Ecosyst* 20:953-969
- Oliveira JM, Brinati A, Miranda LDL, Morais DB, Zanuncio JC, Goncalves RV, Peluzio MDG, Freitas MB (2017) Exposure to the insecticide endosulfan induces liver morphology stress in fruit-eating bats short running title: endosulfan exposure in fruit bats (*Artibeuslituratus*). *Int J Exp Pathol* 98:17-25
- Oliveira JM, Losano NF, Condessa SS, de Freitas RMP, Cardoso SA, Freitas MB, Oliveira LL (2018) Exposure to deltamethrin induces oxidative stress and decreases of energy reserve in tissues of the Neotropical fruit-eating bat *Artibeuslituratus*. *Ecotoxicol Environ Saf* 148:684-692
- Rodhouse TJ, Ormsbee PC, Irvine KM, Vierling LA, Szewczak JM, Vierling KT (2012) Assessing the status and trend of bat populations across broad geographic regions with dynamic distribution models. *Ecol Appl* 22:1098-1113
- Russo D, Ancillotto L (2015) Sensitivity of bats to urbanization: a review. *Mamm Biol* 80:205-212
- Salvarina I (2016) Bats and aquatic habitats: a review of habitat use and anthropogenic impacts. *Mamm Rev* 46:131-143
- Souto HN, de Campos EO, Campos CF, Rodrigues TS, Pereira BB, Morelli S (2018) Biomonitoring birds: the use of a micronuclei test as a tool to assess environmental pollutants on coffee farms in southeast Brazil. *Environ Sci Pollut Res* 25:24084-24092
- Sueitt APE, Yamada-Ferraz TM, Oliveira AF, Botta CMR, Fadini PS, Nascimento MRL, Faria BM, Mozeto AA (2015) Ecotoxicological risks of calcium nitrate exposure to freshwater tropical organisms: laboratory and field experiments. *Ecotoxicol Environ Saf* 117:155-163
- Stahlschmidt P, Hahn M, Brühl CA (2017) Nocturnal risks-high bat activity in the agricultural landscape indicates potential pesticide exposure. *Front Environ Sci* <https://doi.org/10.3389/fenvs.2017.00062>
- Streit B, Nagel A (1993) Heavy metal transfer by lactation in a bat colony. *Fresenius Environ Bull* 2:168-173

- Valdespino C, Sosa VJ (2017) Effect of landscape tree cover, sex and season on the bioaccumulation of persistent organochlorine pesticides in fruit bats of riparian corridors in eastern Mexico. Chemosphere 175:373-382
- Vieira CED, Costa PG, Lunardelli B, de Oliveira LF, Cabrera LD, Risso WE, Primel EG, Meletti PC, Fillmann G, Reis Martinez CB (2016) Multiple biomarker responses in *Prochiloduslineatus* subjected to short-term in situ exposure to streams from agricultural areas in southern Brazil. Sci Total Environ 542:44-56
- Voigt CC, Currie SE, Fritze M, Roeleke M, Lindecke O (2018) Conservation strategies for bats flying at high altitudes. Bioscience 68:427-435
- Walker LA, Bailey LJ, Shore RF (2002) The importance of the gut and its contents in prey as a source of cadmium to predators. Environ Toxicol Chem 21:76-80
- Walker LA, Simpson VR, Rockett L, Wienburg CL, Shore RF (2007) Heavy metal contamination in bats in Britain. Environ Pollut 148:483-490
- Zocche JJ, Leffa DD, Damiani AP, Carvalho F, Mendonca RA, Dos Santos CEI, Boufleur LA, Dias JF, Andrade VM (2010) Heavy metals and DNA damage in blood cells of insectivore bats in coal mining areas of Catarinense coal basin. Environ Res 110:684-691
- Zukal J, Pikulla J, Bandouchova H (2015) Bats as bioindicators of heavy metal pollution: history and prospect. Mamm Biol 80:220-227

CAPÍTULO 2

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Evaluation of genotoxicity in bat species found on agricultural landscapes of the Cerrado savanna, central Brazil[☆]

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Abstract

Habitat loss and fragmentation together represent the most significant threat to the world's biodiversity. In order to guarantee the survival of this diversity, the monitoring of bioindicators can provide important insights into the health of a natural environment. In this context, we used the comet assay and micronucleus test to evaluate the genotoxic susceptibility of 126 bats of eight species captured in soybean and sugarcane plantation areas, together with a control area (conservation unit) in the Cerrado savanna of central Brazil. No significant differences were found between the specimens captured in the sugarcane and control areas in the frequency of micronuclei and DNA damage (comet assay). However, the omnivore *Phyllostomus hastatus* had a higher frequency of nuclear abnormalities than the frugivore *Carollia perspicillata* in the sugarcane area. Insectivorous and frugivorous bats presented a higher frequency of genotoxic damage than the nectarivores in the soybean area. In general, DNA damage and micronuclei were significantly more frequent in agricultural environments than in the control area. While agricultural development is an economic necessity in developing countries, the impacts on the natural landscape may result in genotoxic damage to the local fauna, such as bats. Over the medium to long term, then DNA damage may have an increasingly negative impact on the wellbeing of the local species.

Keywords: Chiroptera, micronucleus test, comet assay, DNA damage, agriculture

1. Introduction

Habitat loss and fragmentation and the expansion of farmland are the principal threats to biodiversity conservation worldwide (Trabaquini et al., 2017; Rocha et al., 2018; Alpizar et al., 2020). In the Cerrado savanna of central Brazil, the encroachment of natural habitats has reached unprecedented levels. Although agriculture is the base of the region's economy, the cultivation of cash crops such as soybean demands the intensive use of pesticides to control pests. Brazil ranks second in the world's major producers and users of pesticides (Martins et al., 2018; Amaral et al., 2021) and still permits the sale of some pesticides banned in many other countries (Medici et al., 2021). Sugarcane is cultivated in many Brazilian states and is the country's principal crop in terms of the production of gross biomass and its third in terms of the area cultivated, after soybean and maize (Bellezoni et al., 2018). Given this scenario, some studies have already focused on the potential impact of the agricultural contaminants in soybean fields on native fauna (Borges et al., 2019; Gonçalves et al., 2019; Rezende et al., 2021) and humans (Ramos et al., 2021). However, few data are available on the effects of sugarcane cultivation on any taxonomic group.

In addition to the changes in the natural landscape caused by large-scale sugarcane production, the application of vinasse, a by-product of the sugarcane milling and distilling processes used as fertilizer (Saad et al., 2017), contaminates the soil and aquatic environment, provoking eutrophication. Vinasse is toxic to both aquatic and terrestrial animals (Christofolletti et al., 2013). Coelho et al. (2017) recorded a significant increase in the immunostaining of the HSP70 stress proteins in the diplopod *Rhinocricus padbergi* in response to exposure to sugarcane vinasse. The genotoxic potential of vinasse was also observed in *Allium cepa* exposed to concentrations of 2.5% and 5%, which induced the formation of nuclear buds, anaphasic bridges, micronucleus, chromosome loss, and chromosome breakage (Garcia et al., 2017).

The fish *Oreochromis niloticus* exposed to different concentrations of vinasse (1%, 2.5%, 5%, and 10%) also showed a dose-dependent response; the higher the concentration of vinasse in the water, the greater the DNA damage in the fish (Correia et al. 2017). Thus, it is suggested that the presence of metals (Ba, Cu, Cr, Ni, Zn, Al, Co, Fe, Mn, and Hg) and low pH of vinasse are the main mechanisms responsible for toxicity (Christofolletti et al., 2013; Garcia et al., 2017). In soils with low pH, metals such as Zn, Cd, Cu, and Pb are released with the degradation of organic matter (de Silva and Souza et al., 2012), which can be clastogenic for some organisms. The evidence thus indicates that the inadequate disposal of vinasse may

represent a potential risk factor for aquatic animals that inhabit bodies of water in the vicinity of sugarcane plantations.

The decline of populations of wild animals likely results from a complex response to a range of stressors at the individual level (Phelps and Kingston, 2018). From this perspective, it is essential to select bioindicators organisms that provide reliable environmental quality diagnosis and meaningful insights for the sustainable development of natural ecosystems. Bats are one potential group of bioindicator organisms, which have been exposed to agricultural, industrial, and urban chemical agents for nearly 50 years in different parts of the world (de Souza et al., 2020). These toxic compounds are known to alter the integrity of the DNA of bats, which respond negatively to the ongoing increase in agricultural land cover and the increasing homogenization of habitats (Put et al., 2019). As bats may forage in or near contaminated plantations, bats may easily assimilate agricultural toxins, affecting their physiology and, ultimately, their ability to provide ecosystem benefits (Sandoval-Herrera et al., 2021). These services include the predation of insect pests, seed dispersal, and pollination. Given this, the present study focused on bat populations' food in soybean and sugarcane growing regions and used a genotoxic approach to evaluate the possible impacts of this farming on these mammals and the potential for predicting the health of natural ecosystems. The analyses were based on examining samples of the exfoliated cells of the oral mucosa and blood of these mammals.

For this ecotoxicological approach, relatively non-invasive, low-cost biomarkers were used –comet assays of peripheral blood cells and the micronucleus (MN) test using exfoliated cells of the oral mucosa– which can elucidate a broad spectrum of DNA damage. Zocche et al. (2010) applied the comet assay successfully to assess insectivorous bats from an area of heavy metal mining in southern Brazil, detecting increased DNA damage compared with other non-mining areas. The micronucleus test is a simple, inexpensive, and minimally invasive technique for evaluating clastogenic or aneugenic damage, and in the present study, this approach will be prioritized due to the overall lack of data on wild mammals. The test is designed to detect aberrations of chromosomal behavior, with micronuclei being formed from the chromatin that does not migrate to the cell poles during anaphase due to chromosome breakage or spindle dysfunction, which results in this material being excluded from the telophase nuclei of the dividing cell (Miller, 1973; Schmid, 1976). These fragments of chromatin, or even entire chromosomes in the case of chromosomal delay, form one or more small nuclei in the cytoplasm of the daughter cells (Miller, 1973). Although micronuclei are recognized universally as a biomarker and have been analyzed in a range of different animal

species, few studies have focused on bats, mainly through the analysis of the exfoliated cells of the oral mucosa (Benvindo-Souza et al., 2019a,b).

Given these considerations, we test the hypothesis that bats captured in areas dominated by soybean and sugarcane plantations have a higher frequency of genotoxic damage than those in a control area (a conservation unit). We also hypothesized that animals from the areas of soybean (harvested annually) have higher rates of genotoxic damage than those from the sugarcane, a semi-perennial crop. The study also aims to provide insights into the use of the micronucleus test on the exfoliated cells of the oral mucosa in wild animals and the comet assay in bats, contributing to the advance of ecotoxicological research of this faunal group in Brazil and other regions around the world (de Souza et al., 2020).

2. Material and Methods

2.1 Sample design and the capture of the bats

The present study was carried out in the southwest of the state of Goiás, in central Brazil. The control area was the Emas National Park (ENP), which covers 132,000 hectares of natural Cerrado savanna vegetation (Fig. 1A; Supplementary Material 1), in the municipalities of Mineiros and Chapadão do Céu, and in Costa Rica municipality in the neighboring state of MatoGrosso do Sul (ICMBio, 2021). The anthropogenic (farmland) areas were selected from forest fragments on soybean/corn farms in the municipality of Rio Verde (Fig. 1B), which have a mean of 19.24% cover of natural vegetation. The sugarcane plantations were located in the municipalities of Paraúna and Acreúna, with 17.92% of natural vegetation cover (Fig. 1C). The two agricultural areas are approximately 70 km apart, and are 215 km (soybean) and 292 km (sugarcane) from the ENP. Bats were collected at five points selected within each of the three areas (Fig. 1; Supplementary Material 1), between 2 km and 40 km apart within an area, to randomize the samples collected in each landscape to minimize their spatial autocorrelation. We consider this minimum distance mainly due to the foraging of some phyllostomids (Heithaus and Fleming, 1987; Aguiar et al., 2014), although they may extrapolate this home range depending on the scarcity of resources.

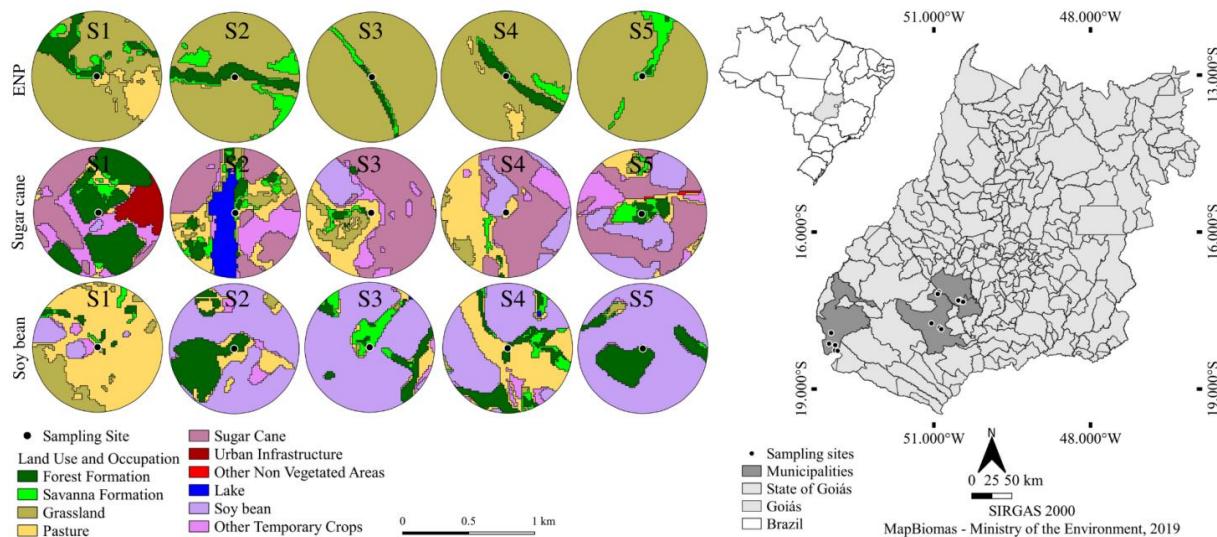


Fig. 1. Sampling points (black circles) and the type of surrounding matrix within a radius of 1 km. Emas National Park (ENP).

The bats were captured using 10 mist-nets, measuring 12 m x 2.5 m, set 50 cm above the ground between sunset and 10 pm over 10 nights in each environment between 2019 and 2021. Thus, they totaled 30 capture nights, and a sampling effort of 36,000 m²/h. The animals collected (all adults) in the nets were placed in cotton bags and then processed to collect biometric data, following Reis et al. (2013), which were used as the criteria for identifying the species. After collecting the blood samples and the exfoliated cells of the oral mucosa, the animals were released at the capture site. To avoid sampling from the same animals, blood was always obtained from the radial artery in the right-wing. If any animal could be recaptured, it would be possible to see the healing in the place where the blood was previously obtained, and the bat would not be sampled.

2.2 Ethical statement

The present study was approved by the Ethics Committee on the Use of Animals of the Federal University of Goiás (n.30/21) and by the Chico Mendes Institute for Biodiversity Conservation (n. 69513-2). No animals were euthanized during the study.

2.3 Analysis of hydrological parameters

Water samples were collected in the three study areas (soybean, sugarcane, and the ENP) for physicochemical analysis and were analyzed within 24 hours of collection. Based on Assis et al. (2021), one liter of water was collected approximately 5 cm below the surface of a body of water close to the sampling point on the same day as the bat captures. The samples

were stored in individual amber borosilicate glass vials at a temperature of less than 4°C and were sent to a private laboratory in Rio Verde, Goiás, Brazil, to quantify their carbamateorganochlorine and organophosphate pesticide content. The following substances were analyzed: 2. 4-D + 2. 4. 5-T, Alachlor; Aldicarb + AldicarbSulfone + AldicarbSulphoxide, Aldrin + Dieldrin, Atrazine, Carbendazim + Benomil, Carbofuran; Chlordane (Cis + Trans), Chlorpyrifos + Chlorpyrifos-Oxon, DDDT, Diuron, Endosulfan (Alpha + Beta + Sulphate), Endrin, Glyphosate + Ampa, Lindane, Mancozeb, Methamidophos, Metolachlor, Molinate, Methylparation, Pendimethalin, Profarmethyl, SimazineTebuconazole, Terbufos, and Trifluralin. These compounds can be found in agricultural areas, either after their recent application or in residual form from previous applications (Assis et al., 2021). The analyses were conducted according to the procedures described in the 23rd edition of the Standard Methods for Examination of Water and Wastewater, as defined by CONAMA (Brazilian Environment Council) Resolution number 357/2005 - Class II (<http://conama.mma.gov.br/>).

2. 4. Comet assays

The alkaline comet assay was based on Singh (1988) with some modifications. Pre-coated slides were prepared with 1.5% standard melting point agarose. Approximately 20 µL of whole blood was obtained from the radial artery of each bat and was diluted in 120 µL of 0.5% low melting point agarose at 37°C. This solution was pipetted onto the coated slides (two per animal), covered with a coverslip. The coverslips were then removed after the solidification of the material, and the slides were incubated at 4°C for 24h in the dark in lysis solution (Triton X-100), using stock lysis (2.5 M sodium chloride, 100 mM disodium salt, 10 mM hydroxymethyl, sodium hydroxide, sodium lauryl sarcosinate) and DMSO. Then the slides were transferred to a horizontal electrophoresis vat and incubated in an alkaline electrophoresis buffer for 30 min. The electrophoretic run was carried out in the dark for 25 min, at 25V and 300mA. After electrophoresis, the slides were neutralized with buffer (0.4 M Tris-HCl, pH 7.5) three times for 5 min, and then washed with cold distilled water and fixed in absolute alcohol for 10 min.

The DNA was stained with 100 µL (10 ng/µL) of the SYBR Gold I solution, and the slides were analyzed in an Imager D2® epifluorescence microscope (Carl Zeiss, Germany), using a 515–560nm excitation filter set for green fluorescence. The slides were analyzed in duplicate for each bat, and 100 nucleoids were counted using the Comet Imager program, version 2.2 (MetaSystems GmbH). All the analyses were conducted by a single researcher

using a 20x lens. Three parameters of genomic damage were evaluated to analyze the nucleoids, based on previous studies (Silva et al., 2020), that is, tail length (TL), the percentage of DNA in the tail (% DNA), and the Olive Tail Moment (OTM).

2.5 Micronucleus tests

Cells were obtained from the oral cavity of the specimens with a flexible cotton-tipped swab, which was rubbed lightly against the lateral cheek mucosa, the bottom of the mouth, and the gums (Benvindo-Souza et al., 2019a,b). Oral mucosa cells were transferred to clean glass slides (four per individual), prepared with a drop of saline solution (0.9% NaCl), which were fixed in a 100% methanol solution for approximately 10 minutes, air-dried before being stained with 5% Giemsa for 10 min and then rinsed with distilled water to remove the excess of the staining solution. Cell counts and photographic documentation (1,000 cells per individual) were conducted on a Lab 1001 TB optical microscope attached to a 3.0 Mp digital camera at a magnification of 100 \times . Micronuclei were defined as structures that (i) are similar to the central nucleus, but no more than one-third of its size, (ii) lack any connection with the central nucleus but have the same (iii) texture, (iv) color intensity, and (iv) rounded or oval shape (following Thomas et al., 2009 and Bolognesi et al., 2013). In addition to micronucleation, other nuclear abnormalities were also evaluated, including the presence of nuclear buds, plasmatic bridging, binucleation, karyorrhexis, pyknosis, and karyolysis (Bolognesi et al., 2013; Benvindo-Souza et al. 2019a,b). The results are expressed as the number of anomalies per 1,000 cells.

2.6 Statistical analysis

The data obtained in the genotoxicity tests are presented as the mean \pm standard error. The normality and homoscedasticity of the data were verified using the Shapiro-Wilk and Levene tests, respectively. Depending on the data distribution, the analyses were based on either the Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test or the Kruskal-Wallis nonparametric analysis of variance (H), with Dunn's *post hoc* test. We compared bat body weights between control and exposed (agricultural) areas for the species found in both, using Student's *t* or Mann-Whitney's *U*. We also verified the potential correlation between body weight and genotoxic variables using the Pearson correlation coefficient. All analyses were run in Statistica v. 7.0, with a $p < 0.05$ significance level.

3. Results

126 bats of eight species were captured in the Cerrado of central Brazil (Fig. 2), representing the families Phyllostomidae, Molossidae, and Vespertilionidae. Peripheral blood samples and exfoliated cells from the oral mucosa of the animals were collected for genotoxic analysis (Fig. 3) to compare control and treatment areas. Native Cerrado vegetation covers 98.5% of the control area, 19.24% of the soybean cultivation area, and 17.92% of the area planted with sugarcane. Generally, the chemical parameters of the collected water samples were below the quantification limits and established by the Brazilian legislation (Supplementary Material 2). The total phosphorus recorded in temporary pools within two sugarcane plantations was three times higher than that recommended for sources of freshwater (0.18 and 0.15 mg/L; Supplementary Material 2).

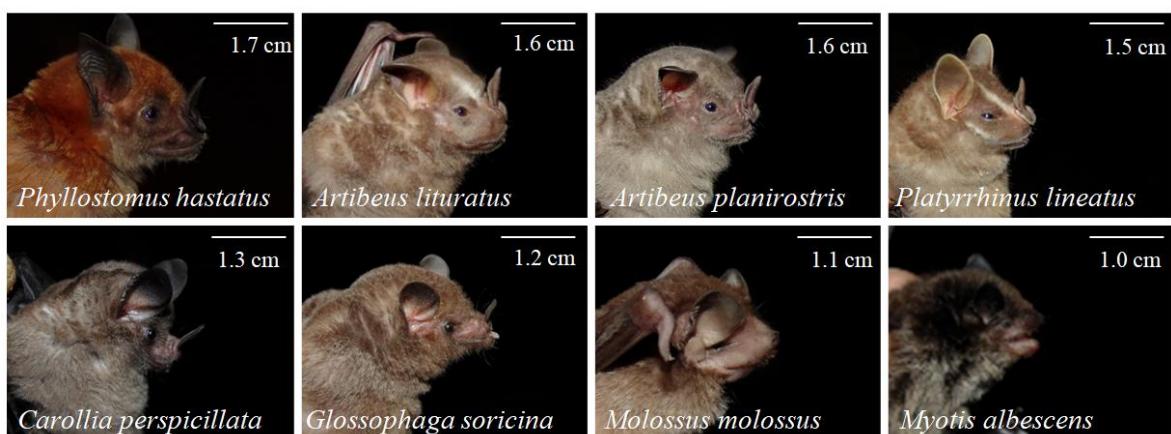


Fig. 2. The eight bat species investigated in the present study using comet assays and the micronucleus test. Size refers to the skull.

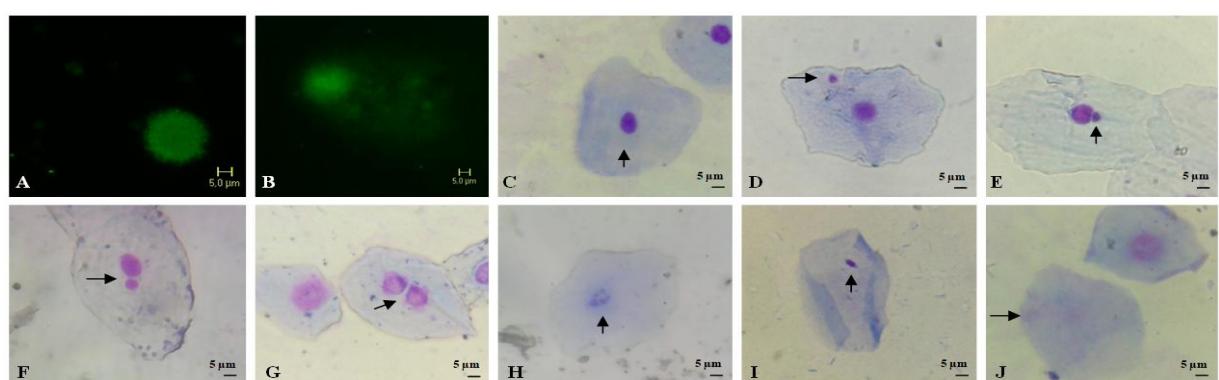


Fig. 3. Photomicrographs showing the nucleoids observed in the comet assay in bats. A) No DNA damage and B) Major DNA damage. Micronucleus test and nuclear abnormalities. C) Normal cell, D) Cell with micronucleus, E) Nuclear buds, F) Plasma bridge in the nucleus, G) Binucleated cell, H) Karyorrhexis, I) Picnosis, and J) Karyolysis. An arrow indicates the nuclear alterations in each case (C-J).

3.1. DNA damage analysis in bats in each environment

The samples from each environment (soybean, sugarcane, and ENP) were evaluated for DNA damage (Table 1). No significant variation was observed among specimens in the control area, and there was also no significant variation in DNA damage among the animals captured in the sugarcane plantation. In the soybean area, the insectivore *Molossus molossus* was significantly different from the nectarivore *Glossophaga soricina* ($H = 20.3$; $p = 0.001$) in the percentage of DNA damage (%DNA). In the case of tail length, *G. soricina*, *M. molossus*, *Carollia perspicillata*, and *Artibeus planirostris* all presented more significant DNA damage than *Artibeus lituratus* ($H = 19.2$; $p = 0.002$). *Molossus molossus* was also significantly different from *A. lituratus* in Olive Tail Moment ($H = 15.3$; $p = 0.009$).

Table 1. The comet assay parameters in bats in each study environment in the Cerrado of central Brazil. The species were compared within each environment (within each column) by multiple comparisons.

Environment/species	Mean ±standard error of the value recorded in the comet assay or micronucleus test		
	%DNA	TL	OTM
ENP			
<i>Glossophaga soricina</i> (n = 12)	11.38±1.33a	13.99±1.77a	1.57±0.23a
<i>Molossus molossus</i> (n = 6)	12.63±2.01a	19.97±2.49a	2.48±0.52a
<i>Carollia perspicillata</i> (n = 9)	16.73±1.89a	15.47±2.50a	2.65±0.55a
<i>Myotis albescens</i> (n = 6)	17.41±4.04a	12.22±2.50a	2.52±0.72a
Sugarcane			
<i>Artibeus lituratus</i> (n = 6)	19.88±3.18a	20.07±3.80a	3.59±0.94a
<i>Carollia perspicillata</i> (n = 12)	34.05±4.30a	29.08±4.43a	6.56±1.18a
<i>Phyllostomus hastatus</i> (n = 12)	26.44±3.85a	21.15±3.02a	5.42±1.03a
<i>Myotis albescens</i> (n = 5)	20.64±3.30a	14.81±1.67a	3.01±0.56a
Soybean			
<i>Artibeus lituratus</i> (n = 7)	25.36±1.45ab	10.25±0.39a	2.56±0.17a

<i>Glossophaga soricina</i> (n = 12)	20.27±2.53a	22.65±3.06b	4.23±0.84ab
<i>Molossus molossus</i> (n = 7)	44.43±8.12b	34.92±7.07b	9.74±2.46b
<i>Carollia perspicillata</i> (n = 11)	21.88±2.26ab	21.11±2.07b	3.52±0.49ab
<i>Platyrrhinus lineatus</i> (n = 9)	25.46±1.92ab	17.36±2.81ab	3.58±0.54ab
<i>Artibeus planirostris</i> (n = 12)	32.79±2.85ab	27.35±4.87b	6.31±1.29ab

Different letters following two values in the same column (per environment) indicate a significant difference ($p < 0.05$) between species (based on the Tukey/ANOVA or Dunn/Kruskal-Wallis procedures), while the same letters indicate that they are statistically similar. %DNA = percentage of DNA in the comet tail; TL = Tail Length; OTM = Olive Tail Moment. Emas National Park (ENP).

3.2. Micronucleus test in species in each environment

The frequency of micronuclei also did not vary significantly among species within each environment (Table 2). However, the sum of the nuclear abnormalities observed in *Phyllostomus hastatus* was significantly greater than that recorded in *C. perspicillata* in the sugarcane plantation ($F = 3.4$; $p = 0.029$). In the soybean area, the sum of nuclear abnormalities was significantly greater in *Artibeus lituratus* compared to *C. perspicillata* ($F = 7.6$; $p = 0.0001$).

Table 2. The mean frequency of micronuclei and other abnormalities recorded in the exfoliated cells of the bat's buccal mucosa in each study environment in the Cerrado of central Brazil. The species were compared within each environment (within each column) by multiple comparisons.

Environment/species	Mean ±standard error of the value recorded in the micronucleus test	
	MN	Total NAs
ENP		
<i>Glossophaga soricina</i> (n = 12)	0.17±0.11a	8.75±1.35a
<i>Molossus molossus</i> (n = 6)	0.17±0.17a	5.65±2.70a
<i>Carollia perspicillata</i> (n = 9)	0.78±0.28a	5.78±0.89a

<i>Myotis albescens</i> (n = 6)	0.33±0.21a	5.67±1.12a
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Sugarcane

<i>Artibeus lituratus</i> (n = 6)	1.17±0.48a	10.00±1.79ab
<i>Carollia perspicillata</i> (n = 12)	1.67±0.40a	10.50±1.56a
<i>Phyllostomus hastatus</i> (n = 12)	1.83±0.30a	18.17±2.67b
<i>Myotis albescens</i> (n = 5)	0.60±0.24a	11.00±1.84ab

Soybean

<i>Artibeus lituratus</i> (n = 7)	1.29±0.42a	21.57±0.93d
<i>Glossophaga soricina</i> (n = 12)	1.42±0.34a	11.58±1.35b
<i>Molossus molossus</i> (n = 7)	2.00±0.58a	12.57±2.93bc
<i>Carollia perspicillata</i> (n = 11)	1.27±0.27a	8.73±1.45ab
<i>Platyrrhinus lineatus</i> (n = 9)	1.22±0.43a	2.89±1.38a
<i>Artibeus planirostris</i> (n = 12)	1.33±0.48a	15.58±2.5cbd

Different letters following two values in the same column (per environment) indicate a significant difference ($p < 0.05$) between species (based on the Tukey/ANOVA or Dunn/Kruskal-Wallis procedures), while the same letters indicate that they are statistically similar. MN = Mincronucleus. NA= Nuclear abnormalities. Emas National Park (ENP).

3.3. Comparison of bat species found in both control and treatment areas (comet assay)

Carollia perspicillata was the most common species in the present study and was sampled in all three environments. The DNA damage recorded in this species was significantly more significant in the sugarcane area in all comet assay parameters than in the control area (Fig. 4A–C). Species such as the nectarivorous *G. soricina* and the insectivorous *M. molossus* and *Myotis albescens* were sampled in at least one of the treatment areas and the control. Genotoxic damage was detected in the *G. soricina* and *M. molossus* samples from the soybean area Fig. 4D–I). However, *M. albescens* did not present any significant difference in the comet assay parameters (Fig. 4J-L).

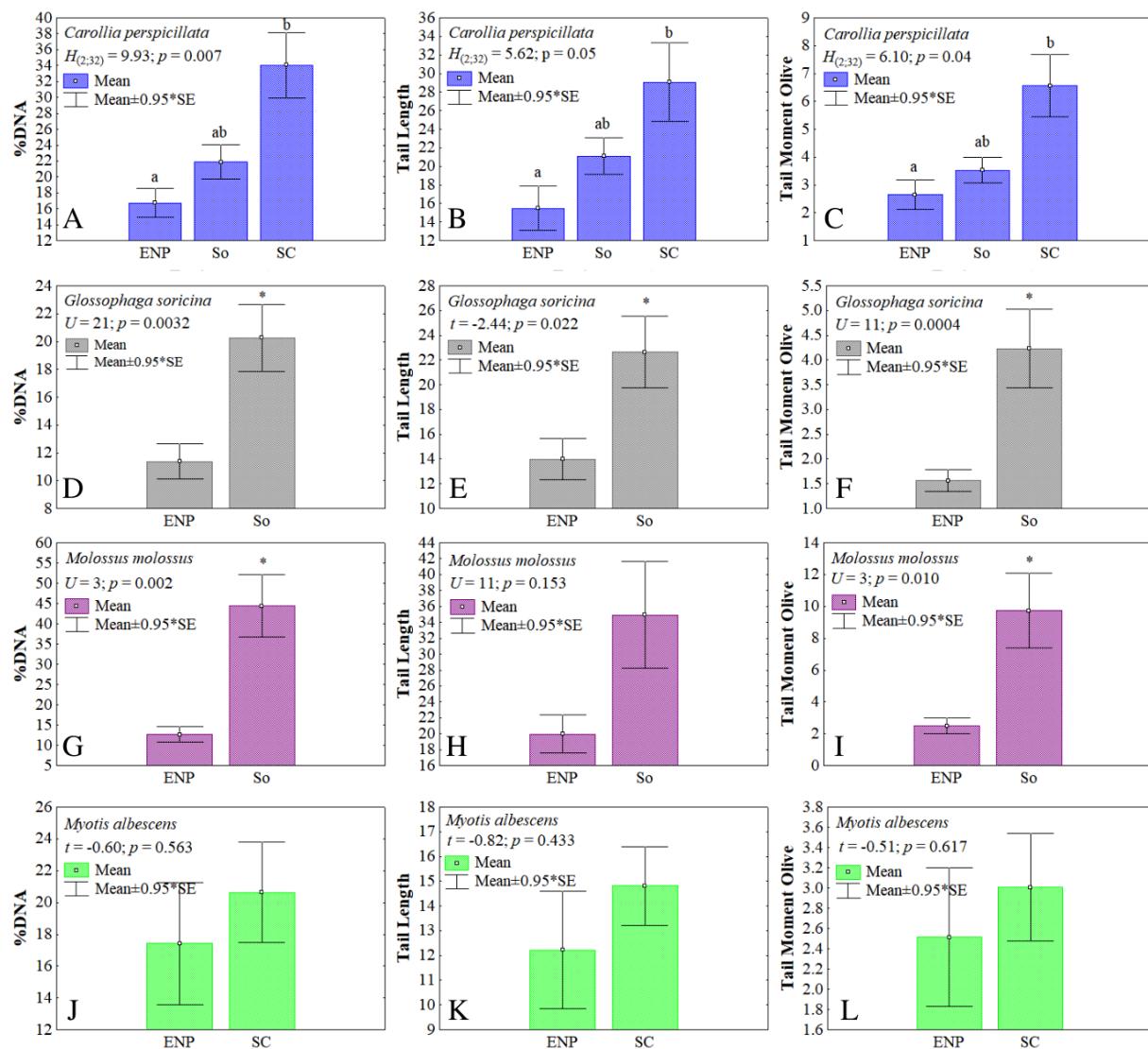


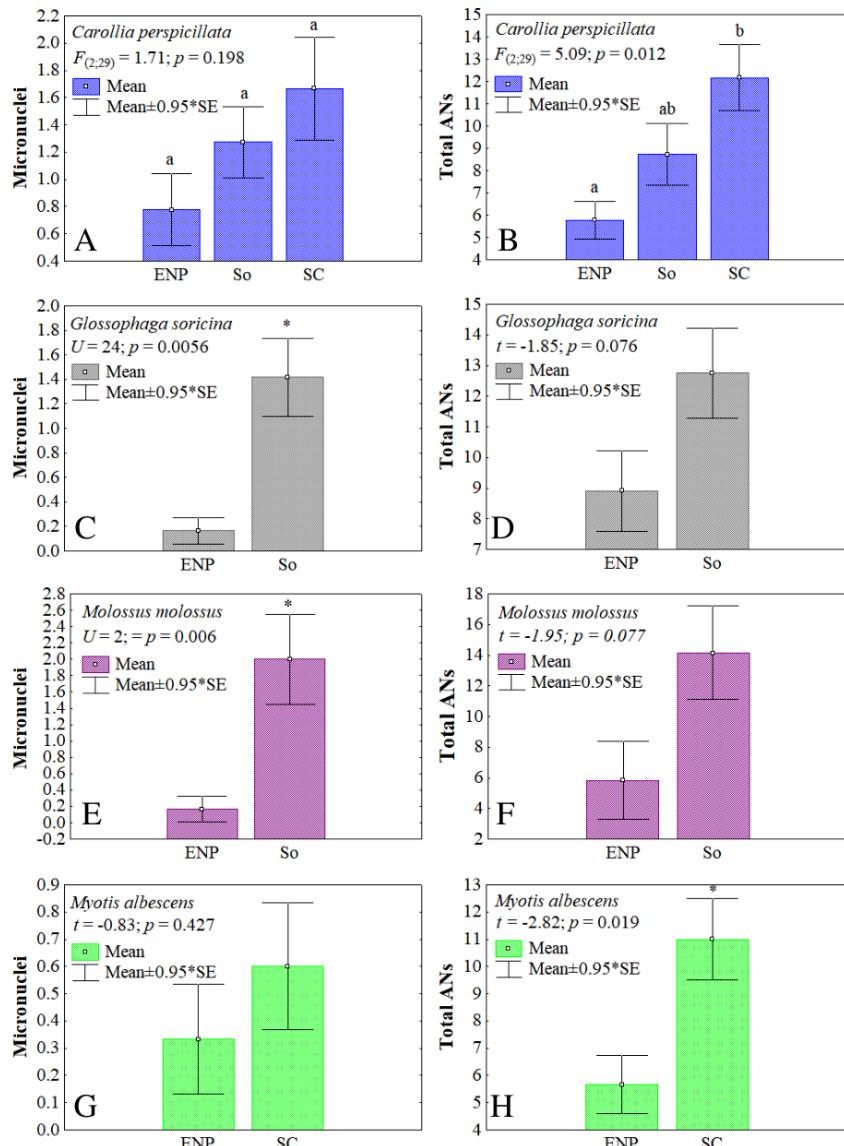
Fig. 4. DNA damage frequency in bats collected in anthropogenic areas (soybean and sugarcane) compared with a control area. ENP = Emas National Park, So = Soybean, and SC = Sugar Cane. Different letters (or asterisk) above columns indicate a significant difference between areas, whereas those marked with the same letters are statistically similar. Kruskal-Wallis, Student's *t* or Mann-Whitney *U* tests were used when appropriate. A *p* < 0.05 level was considered for statistical significance.

3.4. Comparison of bat species found in both control and treatment areas (micronucleus test)

There was no difference in the frequency of micronuclei between the two environments ($F_{(2,29)} = 9.93; p = 0.198$) to *C. perspicillata*. The sum of nuclear abnormalities was greatest in the sugarcane area ($F_{(2,29)} = 5.09; p = 0.012$). Significant differences were also detected in the frequency of micronuclei in *G. soricina* (Fig. 5C; $U = 24; p = 0.006$) and *M. molossus* (Fig. 5E; $U = 2; p = 0.006$) in comparison with the ENP. However, *Myotis albescens* did not present any

significant difference in the micronuclei between the sugarcane areas and the ENP, although there was a significant increase in the frequency in the sum of nuclear abnormalities ($t = -2.82$; $p = 0.019$).

Fig. 5. Mean micronucleus frequency in bat collected from anthropogenic areas (soybean and sugarcane) compared with a control area. ENP = Emas National Park, So = Soybean, and SC = Sugar Cane. Different letters (or asterisk) above columns indicate a significant difference between areas, whereas those marked with the same letters are statistically similar. Kruskal-Wallis, Student's t or Mann-Whitney U tests were used when appropriate. A $p < 0.05$ level was considered for statistical significance.



3.5. Body weight and genotoxicity

The *G. soricina* specimens from the control area had a significantly greater body weight, on average, in comparison with those from the soybean area ($t = 3.1727$, $p = 0.004$; Table 3). The mean body of *M. albescens* was also significantly greater in the control area than the sugarcane area ($t = 4.2851$; $p = 0.002$). No significant variation was found in either *C. perspicillata* or *M. molossus*.

Table 3. Comparison of the mean body weights of the four bat species captured in the control area (Emas National Park) with those recorded in the treatment (agricultural) areas.

Species	Mean ±standard error of the body weight (g) recorded			Results of the analysis
	ENP	Sugarcane	Soybean	
<i>Glossophaga soricina</i>	13.08±0.56*	-	11.00±0.35	$t = 3.1727; p = 0.004$
<i>Molossus molossus</i>	12.67±0.71	-	13.43±0.37	$t = -0.99; p = 0.3434$
<i>Carollia perspicillata</i>	18.56±0.67	17.92±0.67	18.64±0.59	$F_{(2,29)} = 0.3952; p = 0.682$
<i>Myotis albescens</i>	6.83±0.17*	5.60±0.24	-	$t = 4.2851; p = 0.002$

*Significant difference between areas. Emas National Park (ENP).

No significant correlation was found between body weight and any genotoxic parameters in either *G. soricina* or *M. albescens*. However, a significant correlation was found between the body weight of *Molossus molossus* in the sugarcane area and both the micronucleus frequency ($r = -0.8052; p = 0.028$) and DNA damage (Tail Length; $r = 0.7672; p = 0.044$). In *C. perspicillata*, in addition, a negative correlation was found between body weight and %DNA ($r = -0.6479; p = 0.031$) and Olive Tail Moment ($r = -0.6678; p = 0.024$) in the animals from the soybean area. A significant correlation was also found between the weight of the bats and the sum of nuclear abnormalities ($r = -0.7053; p = 0.010$) in the sugarcane area.

4. Discussion

The present study is the first to evaluate DNA damage in *G. soricina*, *M. albescens*, *C. perspicillata*, and *P. hastatus* using the comet assay, and it is also the first study to analyze micronuclei in *C. perspicillata*, *M. albescens*, *P. lineatus*, and *P. hastatus* using exfoliated cells of the oral mucosa. The results indicate that genotoxic damage may vary significantly between species in agricultural landscapes in comparison with conserved areas. No significant variation was found in the DNA damage among bat species within the control area and sugarcane plantation. In the soybean area, the insectivore *M. molossus* presented more significant genotoxic damage than the nectarivorous species, which indicates a differentiated response in the species that may be related to their foraging adaptations or their morphological and

physiological attributes. Regarding food habits, insectivorous bats have a great capacity to ingest contaminated insects, which could affect their health.

In the case of the micronucleus test, once again, no significant variation was observed among species within the environments, except in the case of *M. molossus* in the soybean area. The study was based on specimens collected from five sites within each environment, providing a more heterogeneous sample of the study population, which may be a more realistic scenario for *in situ* ecotoxicological analyses based on replicates of the different treatments. However, caution is needed when considering a given type of anthropogenic matrix. A more realistic scenario could have been developed in the present study if an adequate number of bats had been sampled to permit a more systematic analysis of the level of genotoxic damage within each environment, according to the percentage of land use.

In the sugarcane area, the omnivorous *P. hastatus* had significantly more nuclear abnormalities (total damage) than *C. perspicillata*. Higher frequencies of abnormality were also observed in *A. lituratus*, *M. molossus*, and *A. planirostris* in the soybean area compared to the other species, especially *P. lineatus*. These findings further reinforce our previous conclusions that these three species are promising candidates for biomonitoring in agricultural areas (Benvindo-Souza et al., 2019b). Our study is only the third to apply the micronucleus test to the exfoliated cells of bats as a biomarker for conservation or other applications (Benvindo-Souza et al., 2019a,b). In this way, exfoliated cells are additional merit compared to using only blood.

Previous studies of micronuclei in bats have focused primarily on peripheral blood samples, as in Zuniga-Gonzalez et al. (2000) study of spontaneous micro nucleation in *Artibeus jamaicensis*. Meehan et al. (2004) found an increase in the frequency of micronuclei and DNA damage in *Rhinolophus capensis* in response to low doses of ionizing radiation. Naidoo et al. (2015) found that *Nycteris nana* was sensitive to contact with wastewater in sewage treatment areas. An increased micronuclei frequency was also observed in bats of a site contaminated with mercury (Carlao-Ramos et al., 2021). Olopade et al. (2020) recently applied the micronucleus test to analyze the bone marrow of *Eidolon helvum* and found an increase in both micronucleated polychromatic erythrocytes and normochromatic erythrocytes, indicating genotoxic and cytotoxic responses, respectively. Sandoval-Herrera et al. (2021) also recorded a higher frequency of micronuclei in epithelial blood samples of the insectivore *Pteronotus mexicanus* associated with agricultural activities compared to a control area. Our research group has also successfully evaluated DNA damage in urban populations of *Nyctinomops laticaudatus*, *Noctilio albiventris*, and *Pteronotus parnellii* by analyzing exfoliated cells of the

oral mucosa (Benvindo-Souza et al., 2019a). This study also found a significant difference in DNA damage between animals from agricultural areas in comparison with urban parks (Benvindo-Souza et al., 2019b).

Species found in the agricultural matrix and the control area (*G. soricina* and *M. molossus*) presented significantly more genotoxic damage in the former area. The higher frequency of genotoxic damage in the insectivorous *M. molossus* in the soybean area may be associated with the biomagnification effect of pesticides in the region, considering that the bats were collected primarily in areas with a history of pesticide use and human intoxication (Dutra and Souza, 2017). A similar process cannot be ruled out in the case of *G. soricina* sampled in the soybean matrix, given that, while this species is nectarivorous, it also often consumes insects, especially during periods of resource scarcity in the Cerrado. These findings reinforce the importance of protected areas and highlight the susceptibility of bat species in landscapes dominated by agriculture. Our results are also consistent with the findings of several previous genotoxic studies in the region on different taxa, including amphibians, for example, in which more significant genotoxic damage was detected in areas of soybean cultivation in comparison with more preserved sites (Gonçalves et al., 2017, 2019; Borges et al., 2019; Assis et al., 2021). Studies of birds have also found a higher frequency of genotoxic damage in cropland in comparison with the ENP (Silveira et al., 2021).

Although the present study selected agricultural matrices (with soybean or sugarcane plantations), the water samples collected in lakes and streams in each area did not detect pesticide concentrations below the quantification limit. Our results thus indicate that DNA damage needs to be assessed in terms of the overall quality of the landscape and that new criteria may be needed to evaluate the effects of these compounds at different concentrations. In addition, micronucleation is known to occur in the oral mucosa one to three weeks after contact with xenobiotics (Thomas et al., 2009), which suggests that the exposure occurred before the collection of water samples. We also draw attention to the genetic diversity of these animals, which, when considering genetic polymorphisms, can alter the function of enzymes such as paraoxonase 1 or glutathione-S-transferase, which are involved in the metabolism of pesticides (Costa et al., 2019), increasing pesticide-induced oxidative damage through sources of exposure.

Bats typically have relatively large home ranges, which may increase their susceptibility to exposure compared to many other vertebrates of similar size. The home range of *G. soricina* varies from 430 ha to 890 ha, within which these bats may combine short-haul flights of up to 500 m to nearby areas with longer flights of 2–3 km that take them away from

the central nucleus of the home range (Aguiar et al., 2014). Heithaus and Fleming (1987) recorded approximately one mile (1.6 km) in *C. perspicillata* when moving from the daytime roost to feeding areas and then between feeding areas. The insectivorous *M. molossus*, like other bats of the family Molossidae, lives in stable social groups that forage over a vast spatial scale, flying long distances in search of insect prey (Dechmann et al., 2010). The vespertilionid *M. albescens*, also an insectivore, feeds on coleopterans, dipterans, and lepidopterans (Braun et al., 2009). These bats of this family, particularly those of the genus *Myotis*, appear to range over extensive areas, with females moving up to 25 km in a day between the nursery colony and feeding areas when lactating (Arlettaz et al., 1999).

These bats feed on insects near the vinasse irrigation ponds found throughout the plantation or ingest low-quality water from temporary pools or puddles in the sugarcane area. The total phosphorus in the water samples from two sampling points in the sugarcane was three times higher than the limit established by Brazilian legislation, possibly due to the leaking of vinasse. Vinasse is known to be rich in both essential nutrients such as K, P, S, Fe, Mn, Zn, and Cu, but also in heavy metals with genotoxic potentials, such as Cd, Cr, Ni, and Pb (Srivastava et al., 2010; Marinho et al., 2014; Garcia et al., 2017). Given this, the high concentrations of P found in some areas may account for the abnormalities observed in the insectivorous *M. albescens* and the very common *C. perspicillata*, which, although frugivorous, also consumes insects.

Although vinasse exposure is scarce for vertebrate studies, the genotoxicity of this product with a high presence of phosphorus was observed in studies with *Allium cepa* (Srivastava and Jain 2010; de Silva and Souza et al. 2013) and 1.30mg/L in studies with fish, *Oreochromis niloticus* (Correa et al., 2017), and that together with other nutrients and metals at high levels provide greater genotoxic susceptibility. In this area, these species may thus be vulnerable to contact with metallic elements through the consumption of insects. Christofoletti et al. (2013) observed that some vinasse residues, such as arsenic and mercury, probably accumulated in diplopods. Extrapolating this accumulation to other invertebrates would pose a risk for transfer between the food chain and compromise the health of insectivorous bats. These findings are consistent with the conclusion that vinasse may be highly toxic to animals, plants, microbes, and the freshwater microflora (Christofoletti et al., 2013), although further research will be needed to confirm this link in the case of the bat fauna of these environments.

Overall, it is possible to conclude that the degradation of the natural landscape is a potentially major problem for the local wildlife, especially for animals more susceptible to bioaccumulation, such as predatory bats (de Souza et al., 2020). In the present study, we

detected the sensitivity of bats using genotoxic analyses. Although we did not isolate a stressor by analyzing tissue with a bioaccumulative capacity, our data points to these mammals' sensitivity in areas of intense and large-scale agriculture, such as mechanized soybean, corn, and sugarcane plantations. Carlao-Ramos et al. (2021) found that insect-eating bats in wet areas that received wastewater and toxic sediments from illegal mining had a higher frequency of micronuclei and a higher concentration of mercury in the liver and spleen, indicating a strong association between bioaccumulation and genotoxic damage. However, more studies are needed to focus mainly on the guano and hair of the animals that inhabit agricultural areas or on different tissue types. The variable sex can also be analyzed for the relationship between genotoxicity and xenobiotics. In this study, we do not consider this analysis due to the low sample number for some species. These studies should focus on the species of the family Vespertilionidae, which is widely distributed in South America (Moratelli and Oliveira, 2011) but has not been the focus of studies of DNA damage caused by environmental change in Brazil. This perspective will be critical to monitor the increasingly intensive effects of agriculture on the widespread decline in biodiversity (Put et al., 2019).

Finally, the systematic variation in mean body weight was found among environments, with both *G. soricina* and *M. albescens* being heavier in the control area compared to the farmland. Monthly monitoring in these environments would likely provide more conclusive evidence on this variation among individuals of the same species. The recent study of de Brito et al. (2020) found evidence the body weight of phyllostomid bats varies seasonally in the Brazilian Cerrado. However, other environmental stressors, particularly pesticides, are also known to affect the energy reserves of bats (Amaral et al., 2012a,b), which implies a loss of body mass in scenarios in which the animals are contaminated. A systematic relationship was also found between body weight and the frequency of genotoxic damage in *Molossus molossus* in the sugarcane area, whereas in *C. perspicillata*, a similar pattern was observed in the soybean area. Previous studies did not follow a relationship with genotoxicity in bats in the Cerrado, in the case of *G. soricina* and *M. molossus* from the soybean area (Benvindo-Souza et al., 2019). But this was expected, given that the animals were from the same geographic location. For *C. perspicillata*, this is the first report. Additional other studies will be necessary for discussing these variables as an informative predictor.

5. Conclusion

Overall, the results of the present study allow us to infer that landscapes with a low percentage of native vegetation cover may increase the susceptibility of bats to genotoxic impacts. Here, species with distinct feeding adaptations suffered varying levels of DNA damage within the agricultural landscape of the central Brazilian Cerrado, including the nectarivore *G. soricina*, the frugivore *C. perspicillata*, and the insectivores *M. molossus* and *M. albescens*. The hypothesis that *M. molossus* was the most sensitive species in the soybean area was confirmed. By contrast, *C. perspicillata* was the primary indicator species in the sugarcane area. This study indicates common species, such as *G. soricina*, *C. perspicillata*, and *M. molossus*, as appropriate taxa for biomonitoring.

Further research should focus on samples of hair or guano obtained from these bats, particularly during periods of more intense agricultural management. We confirmed that both the micronucleus test and the comet assay are sensitive biomarkers for the biomonitoring of bat populations. The examination of four slides per animal would be strongly recommended for future bat studies based on the micronucleus test, considering that some species have a tiny oral cavity due to their reduced body size, in which case, a more significant number of slides would ensure standardized sampling for the most reliable possible analysis.

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7. Author contributions

M.B.S collected data in the field, analyzed the data, and wrote the manuscript. A.V.H, C.G.A.S., R.A.A., and T.M.A.P assisted in the fieldwork or the analysis of the biomarkers. R.E.B. assisted with fieldwork and provided critical feedback on research design. S.M.P confirmed the identification of the species and contributed to the research design. L.R.S.S and D.M.S reviewed the manuscript before submission.

8. Competing interests

The authors declare that they have no competing interests.

9. References

- Aguiar, L., Bernard, E., Machado, R.B. 2014. Habitat use and movements of *Glossophagasaricina* and *Lonchophylladekeyseri* (Chiroptera: Phyllostomidae) in a Neotropical savannah. *Zoologia* 31, 223-229. <http://dx.doi.org/10.1590/S1984-46702014000300003>
- Amaral, T.S., Carvalho, T.F., Silva, M.C., Goulart, L.S., Barros, M.S., Picanço, M.C., Neves, C.A., Freitas, M.B. 2012b. Metabolic and histopathological alterations in the fruit-eating bat *Artibeuslituratus* induced by the organophosphorous pesticide fenthion. *Acta Chiropt.* 14, 225-232. <https://doi.org/10.3161/150811012X654420>
- Amaral, T.S., Carvalho, T.F., Silva, M.C., Barros, M.S., Picanço, M.C., Neves, C.A., Freitas, M.B. 2012a. Short-term effects of a spinosyn's family insecticide on energy metabolism and liver morphology in frugivorous bats *Artibeuslituratus* (Olfers, 1818). *Braz. J. Biol.* 72, 299-304. <https://doi.org/10.1590/S1519-69842012000200010>
- Amaral, D.F., Ferreira, J.B.D., Chagas, A.L.S., Adami, M. 2021. Expansion of soybean farming into deforested areas in the amazon biome: the role and impact of the soy moratorium. *Sustain. Sci.* <https://doi.org/10.1007/s11625-021-00942-x>
- Alpizar, P., Schneider, J., Tschapka, M. 2020. Bats and bananas: Simplified diet of the nectar-feeding bat *Glossophagasaricina* (Phyllostomidae: Glossophaginae) foraging in Costa Rican banana plantations. *Glob. Ecol. Conserv.* 24, e01254. <https://doi.org/10.1016/j.gecco.2020.e01254>
- Arlettaz, R. 1999. Habitat selection as a major resource partitioning mechanism between the two sympatric sibling bat species *Myotismyotis* and *Myotis blythii*. *J. Anim. Ecol.* 68, 460-471. <https://doi.org/10.1046/j.1365-2656.1999.00293.x>
- Assis, R.A., Rezende, W.R., Dos Santos, C.G.A. Benvindo-Souza, M., Amorim, N.P.L., Borges, R.E., Franco-Belussi, L., De Oliveira, C., de Souza Santos, L.R., 2021. Habitat differences affect the nuclear morphology of the erythrocytes and the hepatic melanin in *Leptodactylusfuscus* (Anura) in the Brazilian Cerrado savanna. *Environ. Sci. Pollut. Res.* 24. <https://doi.org/10.1007/s11356-021-14974-4>
- Bellezoni, R.A., Sharma, D., Villela, A.A., Junior, A.O.P. 2018. Water-energy-food nexus of sugarcane ethanol production in the state of Goiás, Brazil: An analysis with regional input-output matrix. *Biomass Bioenergy* 115, 108-119. <https://doi.org/10.1016/j.biombioe.2018.04.017>

- Benvindo-Souza, M., Borges, R.E., Pacheco, S.M., Santos, L.R.D. 2019a. Genotoxicological analyses of insectivorous bats (Mammalia: Chiroptera) in central Brazil: The oral epithelium as an indicator of environmental quality. Environ. Pollut. 245, 504-509. <https://doi.org/10.1016/j.envpol.2018.11.015>
- Benvindo-Souza, M., Borges, R.E., Pacheco, S.M., Santos, L.R.S. 2019b. Micronucleus and other nuclear abnormalities in exfoliated cells of buccal mucosa of bats at different trophic levels. Ecotoxicol. Environ. Saf. 172, 120-127. <https://doi.org/10.1016/j.ecoenv.2019.01.051>
- Bolognesi, C., Knasmueller, S., Nersesyan, A., Thomas, P., Fenech, M. 2013. The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay –An update and expanded photogallery. Mutat. Res. Rev. Mutat. Res. 753, 100-113. <https://doi.org/10.1016/j.mrrev.2013.07.002>
- Borges, R.E., de Souza Santos, L.R., Benvindo-Souza, M., Modesto, R.S., Assis, R.A., de Oliveira, C. 2019. Genotoxic evaluation in tadpoles associated with agriculture in the Central Cerrado, Brazil. Arch. Environ. Contam. Toxicol. 77, 22-28. <https://doi.org/10.1007/s00244-019-00623-y>
- Braun, J.K., Layman, Q.D., Mares, M.A. 2009. *Myotis albescens* (Chiroptera: Vespertilionidae). Mamm. Species 25, 1-9. <https://doi.org/10.1644/846.1>
- Calao-Ramos, C., Gaviria-Angulo, D., Marrugo-Negrete, J., Calderón-Rangel, A., Guzmán-Terán, C., Martínez-Bravo, C., Mattar, S. 2021. Bats are an excellent sentinel model for the detection of genotoxic agents. Study in a Colombian Caribbean region. Acta Trop. 106141. <https://doi.org/10.1016/j.actatropica.2021.106141>
- Coelho, M.P.M., Moreira-de-Sousa, C., de Souza, R.B., Ansoar-Rodríguez, Y., Silva-Zacarin, E. C. M., Fontanetti, C. S. 2017. Toxicity evaluation of vinasse and biosolid samples in diplopod midgut: heat shock protein in situ localization. Environ. Sci. Pollut. Res. 24, 22007–22017. <https://doi.org/10.1007/s11356-017-9754-2>
- Correia, J. E., Christofolletti, C. A., Ansoar-Rodríguez, Y., Guedes, T. A., Fontanetti, C. S. 2017. Comet assay and micronucleus tests on Oreochromisniloticus (Perciforme: Cichlidae) exposed to raw sugarcane vinasse and to phisicochemical treated vinasse by pH adjustment with lime (CaO). Chemosphere, 173, 494-501. <https://doi.org/10.1016/j.chemosphere.2017.01.025>
- Christofolletti, C.A., Escher, J.P., Correia, J.E., Marinho, J.F.U., Fontanetti, C.S. 2013. Sugar cane vinasse: environmental implications of its use. Waste Manag. 33, 2752-2761. <https://doi.org/10.1016/j.wasman.2013.09.005>

- Christofoletti, C.A., Pedro-Escher, J., Fontanetti, C.S. 2013. Assessment of the genotoxicity of two agricultural residues after processing by diplopods using the Allium cepa assay. Water Air Soil Pollut. 224, 1-14. <https://doi.org/10.1007/s11270-013-1523-3>
- da Silva, E.P., Benvindo-Souza, M., Cotrim, C.F.C., Motta, A.G.C., Lucena, M.M., AntoniosiFilho, N.R., Pereira, P., Formiga, K.T.M., Silva, D.M. (2020). Genotoxic effect of heavy metals on *Astyanaxlacustris* in an urban stream. Heliyon 6, e05034. <https://doi.org/10.1016/j.heliyon.2020.e05034>
- da Silva Souza, T., Hencklein, F. A., de Angelis, D. D. F., Fontanetti, C. S. 2013. Clastogenicity of landfarming soil treated with sugar cane vinasse. Environ. Monit. Assess. 185, 1627-1636. <https://doi.org/10.1007/s10661-012-2656-3>
- de Souza, M.B., de Souza Santos, L.R., Borges, R.E., Nunes, H.F., Vieira, T.B., Pacheco, S.M., Silva, D.D.M. 2020. Current Status of Ecotoxicological Studies of Bats in Brazil. Bull. Environ. Contam. Toxicol. 104, 393-399. <https://doi.org/10.1007/s00128-020-02794-0>
- de Brito, J.L.M., Amaral, T.S., Aguiar, L.M.D.S., Lucci, C.M. 2020. Evaluation of reproductive parameters in male Neotropical bats during dry and rainy months in a specific area of the Cerrado biome. Anat. Histol. Embryol. 49, 307-314. <https://doi.org/10.1111/ahe.12529>
- Dechmann, D.K.N., Kranstauber, B., Gibbs, D., Wikelski, M. 2010. Group Hunting-A Reason for Sociality in Molossid Bats? Plos One 5, e9012. <https://doi.org/10.1371/journal.pone.0009012>
- Dutra, R.M.S., Souza, M.M.O. 2017. Impactos Negativos do Uso de Agrotóxicos à Saúde Humana. Hygeia 13, 127-140. <http://www.seer.ufu.br/index.php/hygeia/article/viewFile/34540/20580>
- Garcia, C.F.H, de Souza, R.B., de Souza, C.P., Christofoletti, C.A., Fontanetti, C.S. 2017. Toxicity of two effluents from agricultural activity: Comparing the genotoxicity of sugar cane and orange vinasse. Ecotoxicol. Environ. Saf. 142, 216-221. <http://orcid.org/10.1016/j.ecoenv.2017.03.053>
- Gonçalves, M.W., Gambale, P.G., Godoy, F.R., Alves, A.A., Rezende, P.H.D., Maciel, N.M., Nomura, F., Bastos, R.P., Marco, P. 2017. The agricultural impact of pesticides on *Physalaemus cuvieri* tadpoles (Amphibia: Anura) ascertained by comet assay. Zoologia. 4, e19865. <http://zoobank.org/A65FFC07-75B6-4DE4-BE59-8CE6BB2D4448>
- Gonçalves, M.W., de Campos, C.B.M., Godoy, F.R., Gambale, P.G., Nunes, H.F., Nomura, F., Bastos, R.P., Cruz, A.D., Silva, D.D.M. 2019. Assessing genotoxicity and mutagenicity of

- three common amphibian species inhabiting agroecosystem environment. Arch. Environ. Contam. Toxicol. 77, 409-420. <https://doi.org/10.1007/s00244-019-00647-4>
- Heithaus, E.R., Fleming, T.H. 1978. Foraging movements of a frugivorous bat, *Carollia perspicillata* (Phyllostomatidae). Ecol. Monogr. 48, 127-143. <https://doi.org/10.2307/2937296>
- ICMBio. 2019. Instituto Chico Mendes de Conservação da Biodiversidade. Parque Nacional das Emas. Available in: <https://www.gov.br/icmbio/pt-br/assuntos/biodiversidade/unidade-de-conservacao/unidades-de-biomas/cerrado/lista-de-ucs/parna-das-emas/informacoes-sobre-visitacao-2013-parna-das-emas>. Accessed July 2021.
- Marinho, J.F.U., Correia, J.E., de Castro Marcato, A.C., Pedro-Escher, J., Fontanetti, C.S. 2014. Sugar cane vinasse in water bodies: Impact assessed by liver histopathology in tilapia. Ecotoxicol. Environ. Saf. 110, 239-245. <https://doi.org/10.1016/j.ecoenv.2014.09.010>
- Martins, E.H., Vilela, A.P., Mendes, R.F., Mendes, L.M., Vaz, L.E.V.D.B., Guimaraes, J.B. 2018. Soybean waste in particleboard production. Cienc. Agrot. 42, 186-194. <https://doi.org/10.1590/1413-70542018422015817>
- Medici, E.P., Fernandes-Santos, R.C., Testa-Jose, C., Godinho, A.F., Brand, A.F. 2021. Lowland tapir exposure to pesticides and metals in the Brazilian Cerrado. Wildl. Res. 48, 393-403. <http://orcid.org/10.1071/WR19183>
- Meehan, K.A., Truter, E.J., Slabbert, J.P., Parker, M.I. 2004. Evaluation of DNA damage in a population of bats (Chiroptera) residing in an abandoned monazite mine. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 557, 183-190. <http://orcid.org/10.1016/j.mrgentox.2003.10.013>
- Miller, R.C. 1973. The micronucleus test as an in vivo cytogenetic method. Environ. Health. Perspect. 6, 167-170. <https://ehp.niehs.nih.gov/doi/pdf/10.1289/ehp.7306167>
- Moratelli, R., Oliveira, J.A. 2011. Morphometric and morphological variation in South American populations of *Myotis albescens* (Chiroptera: Vespertilionidae). Zoologia. 28, 789-802. <https://doi.org/10.1590/S1984-46702011000600013>
- Naidoo, S., Vosloo, D., Schoeman, M.C. 2015. Haematological and genotoxic responses in an urban adapter, the banana bat, foraging at wastewater treatment works. Ecotoxicol. Environ. Saf. 114, 304-311. <https://doi.org/10.1016/j.ecoenv.2014.04.043>
- Olopade, J.O., Anosike, F., Lanipekun, D.O., Adebiyi, O.E., Ogunsuyi, O.M., Bakare, A.A. 2020. Haematological Studies and Micronucleus Assay of Straw-Coloured Fruit Bats (*Eidolon helvum*). Niger. J. Physiol. Sci. 35, 181-186.

- Put, J.E., Fahrig, L., Mitchell, G.W. 2019. Bats respond negatively to increases in the amount and homogenization of agricultural land cover. *Landsc. Ecol.* 34, 1889-1903. <http://orcid.org/10.1007/s10980-019-00855-2>
- Phelps, K.L., Kingston, T. 2018. Environmental and biological context modulates the physiological stress response of bats to human disturbance. *Oecologia* 188, 41-52. <https://doi.org/10.1007/s00442-018-4179-2>
- Ramos, J.S.A., Pedroso, T.M.A., Godoy, F.R., Batista, R.E., de Almeida, F.B., Francelin, C., Ribeiro, F.L., Parise, M.R., Silva, D.D.M. 2021. Multi-biomarker responses to pesticides in an agricultural population from Central Brazil. *Sci. Total. Environ.* 754, 141893. <https://doi.org/10.1016/j.scitotenv.2020.141893>
- Reis, N.R., Fregonezi; M.N., Peracchi, A.L., Shibatta, O.A. 2013. Morcegos do Brasil: Guia de Campo. Rio de Janeiro: Technical Books. Londrina.
- Rezende, W.R., de Souza Santos, L.R., Franco-Belussi, L., De Oliveira, C. 2021. Testicular morphometric changes in neotropical anurans from agroecosystems. *Environ. Pollut.* 271, 116265. <https://doi.org/10.1016/j.envpol.2020.116265>
- Rocha, E.C., Brito, D., Silva, P.M., Silva, J., Bernardo, P.V.S., Juen, L. 2018. Effects of habitat fragmentation on the persistence of medium and large mammal species in the Brazilian Savanna of Goiás State. *Biota Neotrop.* 18, e20170483. <https://doi.org/10.1590/1676-0611-BN-2017-0483>
- Thomas, P., Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S., Fenech, M. 2009. Buccal micronucleus cytome assay. *Nat. Protoc.* 4, 825. <https://doi.org/10.1038/nprot.2009.53>
- Trabaquini, K., Galvao, L.S., Formaggio, A.R., de Aragao, L.E.O.E.C. 2017. Soil, land use time, and sustainable intensification of agriculture in the Brazilian Cerrado region. *Environ. Monit. Assess.* 189:2. <https://doi.org/10.1007/s10661-017-5787-8>
- Saad, L.P., Souza-Campana, D.R., Bueno, O.C., Morini, M.S.C. 2017. Vinasse and its influence on ant (Hymenoptera: Formicidae) communities in sugarcane crops. *J. Insect Sci.* 17, 11. <https://doi.org/10.1093/jisesa/iew103>
- Sandoval-Herrera, N., Castillo, J.P., Montalvo, L.G.H., Welch, K.C. 2021. Micronucleus test reveals genotoxic effects in bats associated with agricultural activity. *Environ. Toxicol. Chem.* 40, 202-207. <https://doi.org/10.1002/etc.4907>
- Silveira, E.D.R., Benvindo-Souza, M., Assis, R.A., Dos Santos, C.G.A., de Lima Amorim, N.P., Borges, R. E., Melo, C., de Souza Santos, L.R. 2021. Micronucleus and different

nuclear abnormalities in wild birds in the Cerrado, Brazil. Environ. Sci. Pollut. Res. <https://doi.org/10.1007/s11356-021-16845-4>

Singh, S., Kumar, V., Singh, P., Banerjee, B.D., Rautela, R.S., Grover, S.S., Rawat, D.S., Pasha, S.T., Jain, S.K., Rai, A. 2012. Influence of CYP2C9, GSTM1, GSTT1 and NAT2 genetic polymorphisms on DNA damage in workers occupationally exposed to organophosphate pesticides. Mutat. Res. 741, 101-108. <https://doi.org/10.1016/j.mrgentox.2011.11.001>

Schmid, W. 1976. The Micronucleus Test for Cytogenetic Analysis. In: Hollaender A. (eds) Chemical Mutagens. Chemical Mutagens (Principles and Methods for Their Detection). Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-0892-8_2

Srivastava, S, Jain, R. 2010. Effect of distillery spent wash on cytomorphological behaviour of sugarcane settling. J. Environ. Biol. 31, 809. http://www.jeb.co.in/journal_issues/201009_sep10_supp/paper_12.pdf

Zocche, J.J., Leffa, D.D., Damiani, A.P., Carvalho, F., Mendonca, R.A., Dos Santos, C.E.I., Boufleur, L.A., Dias, J.F., Andrade, V.M. 2010. Heavy metals and DNA damage in blood cells of insectivore bats in coal mining areas of Catarinense coal basin, Brazil. Environ. Res. 110, 684-691. <https://doi.org/10.1016/j.envres.2010.06.003>

Zúñiga-González, G., Torres-Bugarín, O., Luna-Aguirre, J., González-Rodríguez, A., Zamora-Perez, A., Gómez-Meda, B.C., Ventura-Aguilar, A.J., Ramos-Ibarra, M.L., Ramos-Mora, A., Ortíz, G.G., Gallegos-Arreola, M.P. 2000. Spontaneous micronuclei in peripheral blood erythrocytes from 54 animal species (mammals, reptiles and birds): Part two. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 467: 99-103. [https://doi.org/10.1016/S1383-5718\(00\)00021-8](https://doi.org/10.1016/S1383-5718(00)00021-8)

CAPITULO 3

Genotoxic, mutagenic, and cytotoxic analysis in bats in a Cerrado mining area

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Abstract

Pollution generated by the mining industry can harm wildlife. This study aimed to evaluate the cytotoxicity, genotoxicity and mutagenicity in bats environmentally exposed to open pit mining. The bats were collected in July and November of 2021, totaling 8 sampling days offield work. We obtained 62 bats of the species *Carollia perspicillata*, *Glossophaga soricina*, *Phyllostomus hastatus*, and *Desmodus rotundus*. The results indicated that species differ in the frequency of genotoxic damage between sampling points within the mining landscape. Cytotoxicity was observed by scoring of karyorrhexis, pyknosis and karyolysis. The most captured species, *C. perspicillata*, showed significant differences in DNA damage between exposed and unexposed populations, but no differences were observed between males (n=14) and females (n=20). *G. soricina* was also a sensitive species for indicating a high frequency of DNA damages compared to the omnivore *P. hastatus*. Elements such as Mn, Cr, Pb, and Zn observed in water samples were at high levels in the mining area. We conclude that bats in mining areas are susceptible to increased DNA damage as already confirmed in other studies.

Keywords: Chiroptera; metals; DNA damage; comet assay; micronucleus test

1. Introduction

Pollution by metals and semimetals is one of the biggest threats to ecosystems, because it degrades habitat and is toxic to wildlife and human population (Ferrante et al. 2017). Thus, it is necessary to monitor potentially polluting areas such as mining. Ore extraction is known to pollute natural resources in many parts of the world (Tovar-Sánchez et al. 2012). Many organisms that inhabit industrial areas are exposed to high concentrations of metals and can suffer chronic poisoning if, for example, metallic elements are transferred to food (Flache et al. 2016). According to Zukal et al. (2015), eleven elements that concern wildlife are arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, tin, and thallium. Nickel, cadmium, and chromium are considered carcinogenic, while aluminum, lead, and tin are neurotoxic (Kozlowski et al. 2014). Nickel (Ni) is a versatile compound used in a wide variety of industrial processes produced worldwide (Tognacchini et al. 2020). Anthropogenic nickel release to the environment occurs locally from mining, smelting, and metal refining operations; industrial activities, such as nickel-plating and alloy manufacturing; sludge, solids, and slag on land; and disposal as effluents (Buxton et al. 2019).

According to Annangi et al. (2016), nickel exists in several forms, including elemental nickel (Ni), nickel oxide (NiO), nickel chloride (NiCl₂), nickel sulfate (NiSO₄), nickel carbonate (NiCO₃), nickel monosulfide (NiS) and nickel subsulfide (Ni₃S₂). Nickel is among the metals widely used in many important applications, including buildings and infrastructure, transportation, industrial machinery, household appliances (Elshkaki et al. 2017), and personal items such as utensils and jewelry (Rizvi et al. 2020). Brazil currently ranks the second in Ni reserves, with the largest one located in the State of Goiás (77.2%) (Silva and Braga 2020).

Although the country stands out in mining, few toxicological and ecotoxicological studies have been carried out in Ni exploration areas. In the Goias state, a single study was carried out with anuran amphibians indicating genotoxic and mutagenic responses to Ni mining (Gonçalves et al. 2012). Sotero (2022) evaluated the sensitivity of two bat species, *Glossophaga soricina*, and *Carollia perspicillata*, in the same iron/nickel mining area compared to a conservation unit. Thus, there is a necessity of understand the response of other bat species in the region, especially comparing different points along the ore extraction area.

Bats are nocturnal mammals that belong to the order Chiroptera. They fly naturally using a very long outstretched fingers covered by a thin membrane or patagium (Lagunas-Rangel 2020). Some species are over 20 years old, who's most surprising record was the insectivore *Myotis brandtii* at 41 years of age (Podlutsky et al. 2005). These animals are globally distributed and show predictable responses to environmental stressors such as heavy

metals (Zukal et al. 2015; Gallant et al. 2020). The primary exposure source for bats is through ingestion of contaminated food and water, followed by dermal exposure and inhalation (de Souza et al. 2020; Calao-Ramos et al. 2021). Several studies have highlighted the accumulative capacity of metals in bat tissues in the last 50 years and the damage related to toxic elements (Zukal et al. 2015; Carrasco-Rueda et al. 2020; dos Santos Pedroso-Fidelis et al. 2020). Given the sensitivity of bats to xenobiotics, these animals have been used as bioindicators to assess environmental quality (Calao-Ramos et al. 2021; Benvindo-Souza et al. 2022).

In this context, biomarkers are necessary for the environmental assessment of bat species. One example is the comet assay, also known as single-cell gel electrophoresis, being increasingly used to verify the genotoxicity of various xenobiotics (Singh et al. 2019). In addition to the comet assay, the micronucleus test on the oral mucosa exfoliated cells is also a non-invasive method for monitoring populations exposed to genotoxic risks (Benvindo-Souza et al. 2019a; Lopes et al. 2021). For Ni, in studies with mice, this metal was aneuploid, acting on kinetochores, causing chromosomal nondisjunction, being clastogenic (El-Habit and Moneim 2014). Thus, those authors demonstrated that Ni could increase the frequency of aneuploidies, inducing significant DNA damage in mouse bone marrow cells (El-Habit and Moneim 2014). Contact with nickel in humans, for example, can trigger a series of side effects such as allergy, cardiovascular and kidney disease, pulmonary fibrosis, pulmonary and nasal cancer (Genchi et al., 2020) which indicates its toxicity in mammals. Finally, in view of the above, the objective of the present study was to study the frequency of genotoxic, mutagenic and cytotoxic damage of bats of a Cerrado mining activity area.

2. Material and methods

2.1 Environments and the capture of bats

Five environments were selected for sampling bats in Goias State, Brazil (Fig. 1). The captures took place between July and November 2021. Four of the environments were in iron/nickel mining areas; the first two (S1 and S2) are situated in the municipality of Barro Alto, and the third and fourth areas are in the municipality of Niquelândia (S3 and S4). In Barro Alto mining activities have been in operation since 2011, while Niquelândia is one of the largest nickel reserves in the world, with a history of more than 40 years (Strauch et al. 2011). At Barro Alto, a fifth area (reference area) was sampled on the margins of riparian forests, approximately 25 km in a straight line from the mine and without a history of ore exploration. In the sampling areas, they indicated 15.37% of ore exploration at site 1, according to an analysis of a 2km radius. 26.4% on site 2, 10.78 at site 3, and 18.4% at site 4. The

characterization of land use and coverage of the areas (evaluation of vegetation percentage) was performed at QGIS, using a Mapbiomas mesh for the year 2021.

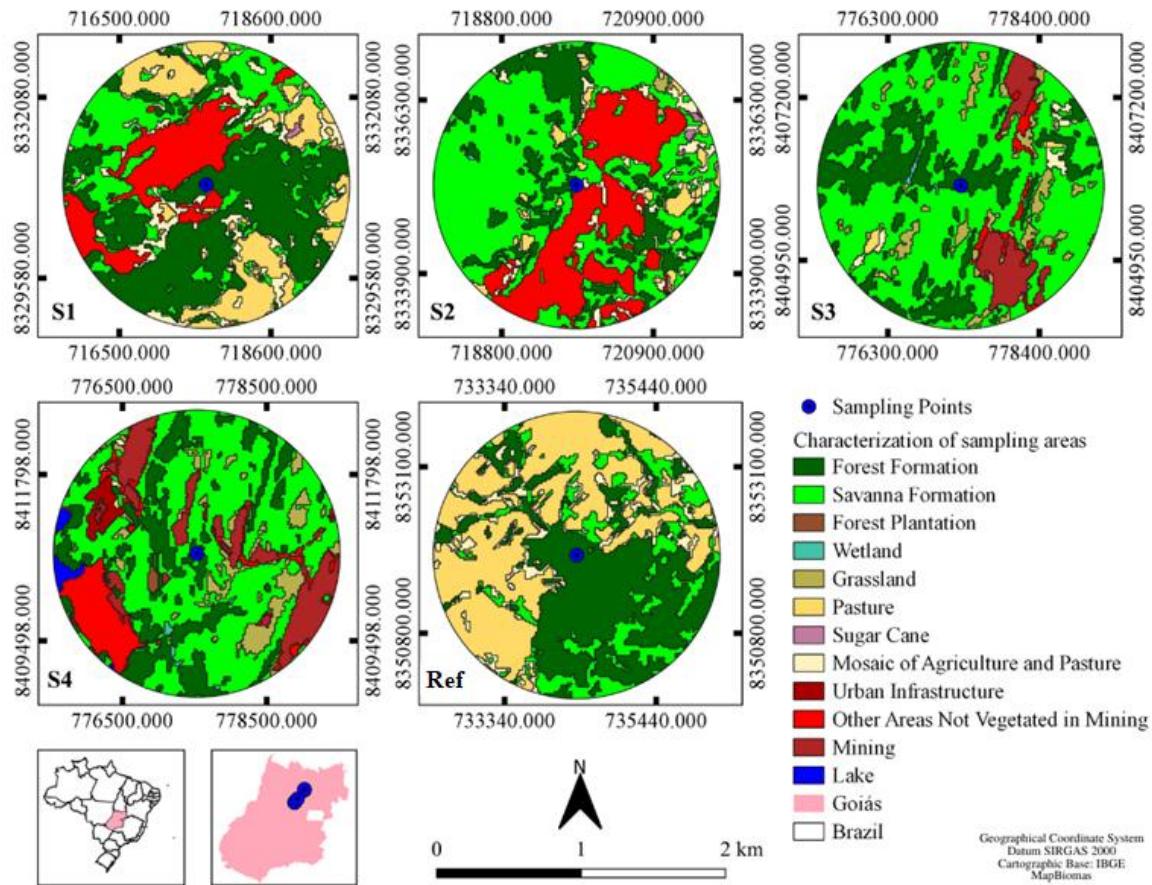


Fig. 1 Sampling sites (centroid in blue) and surrounding matrix type within a 2 km radius in the municipalities of Niquelândia and Barro Alto, Goiás State indicate the first four mining areas, and the fifth point is the reference area, far from mining activity.

Bats were captured using 10 mist nets, measuring 12 m x 2.5 m, 50 cm above ground between 18 to 22h by eight nights in 2021. The sampling effort was 9,600 m²/h. The animals collected in the nets were placed in cotton bags and subsequently processed for sample collection. Pregnant females were only quantified and released at the same capture site, given that handling can lead the animal to abort the offspring (Benvindo-Souza et al. 2019a). The identification of animals was according to Reis et al. (2013). The animals were then released at the capture site after collecting blood samples and exfoliated cells from the oral mucosa. To avoid collecting the same animals, blood was always obtained from the radial artery of the right wing. If any animal could be recaptured, it would be possible to see healing at the site where blood was previously obtained, and the bat would not be resampled (Benvindo-Souza et al. 2022).

2.2 Water analysis

Water samples were collected to analyze Mn, Fe, Al, Cr, Cu, Ni, Pb, Zn, Cd, and Si. First, one liter of water was collected approximately 5 cm below the water body surface close to the sampling point on the same day of the bat capture. The samples were stored in individual vials at a temperature below 4°C and sent to be analyzed at maximum 24 hours after collection to a private laboratory in Rio Verde, Goiás, Brazil, to quantify the metal content.

2.3 Comet assay

The alkaline comet assay was based on Singh (1988) with some modifications (Benvindo-Souza et al. 2022). Pre-coated slides were prepared with standard 1.5% melting point agarose. Approximately 20 µL of whole blood was obtained from the radial artery of each bat and diluted in 120 µL of low melting point 0.5% agarose at 37 °C. This solution was pipetted onto the coated slides (two per animal) and covered with a coverslip. The coverslips were then removed after solidification, and the slides were incubated at 4 °C for 24 h in the dark in lysis solution (stock lysis: 2.5 M sodium chloride, 100 mM disodium salt, 10 mM hydroxymethyl, sodium lauryl sarcosinate), Triton X-100 and DMSO. Then the slides were transferred to a horizontal electrophoresis vat and incubated in alkaline electrophoresis buffer for 30 min. The electrophoretic run was performed in the dark for 25 min, at 25V and 300mA. After electrophoresis, the slides were neutralized with buffer (0.4 M Tris-HCl, pH 7.5) three times for 5 min, then washed with cold distilled water and fixed in absolute alcohol for 10 min. DNA was stained with 100µL (10 ng/ul) of SYBR Gold I solution, and slides were analyzed on an Imager D2® epifluorescence microscope (Carl Zeiss, Germany), using a 515-560nm excitation filter set for fluorescence. Slides were analyzed in duplicate for each bat, and 100 nucleoids were counted using the Comet Imager program, version 2.2 (Meta Systems GmbH). All analyzes were carried out by a single researcher with a 20x objective (or magnification). Three parameters of genotoxic damage were evaluated to analyze the nucleoids, namely, the tail length (TL), the DNA percentage in tail (% DNA), and Olive's Tail Moment (MCO).

2.4 Micronucleus test

The micronucleus test was applied for mutagenicity and cytotoxicity analysis. Cells were obtained from the subjects' oral cavity with a flexible cotton-tipped swab, which was lightly rubbed against the lateral mucosa of the cheek, the back of the mouth, and the gums (Benvindo-Souza et al. 2019a,b, 2022). Oral mucosa cells were transferred to clean glass slides (four per individual), prepared with a drop of saline solution (0.9% NaCl), which were fixed in

100% methanol solution for approximately 10 minutes, dried at the air before being stained with Panoptic Rapid by dipping the slides five times in each solution and then rinsing with distilled water to remove excess dye solution. 1,000 cells per individual were counted under an optical microscope at $\times 100$ magnification. Other nuclear abnormalities were also evaluated, including the presence of nuclear bud (NB), binucleated BI), karyorrhexis (KX), pyknosis (PY), and karyolysis (KY).

2.5 Statistical analysis

The data obtained are presented as mean \pm standard error. The normality and homoscedasticity of the data were verified by the Shapiro-Wilk and Levene tests, respectively. Depending on the data distribution, Analysis of Variance (ANOVA) followed by Tukey's post hoc test or Kruskal-Wallis' non-parametric analysis of variance (H) with Dunn's post hoc test were used. The student's t-test was used for comparisons between male and female animals. All analyses were performed on Statistica v. 7.0, with a $p<0.05$. A similarity Dendrogram (Bray-Curtis Cluster Analysis) between the sampling sites and the total ANs was performed using the PAST software following Gonçalves (2022), with adaptations.

3. Results

3.1 Surface water samples

The chemical analysis of the water in the mining and reference area is shown in Table 1. Four elements of metals Mn, Cr, Pb, and Zn were above the limit established by Brazilian legislation for fresh water. Cr and Pb were observed at sites one and Ref.; Mn, Cr, Pb, and Zn at sites two and four. In site three were observed Mn, Cr, Pb, and Zn. Other elements such as Fe, Al, Cu, and Si do not have a total limit in type I fresh water. On the other hand, the amount dissolved is established for some of these elements as in Fe (0.3 mg/L), Al (1, 5 mg/L), and Cu (0.009 mg/L), which draws attention mainly to the latter.

Table 1 Chemical results of water samples (mg/L) in the mining area and reference in relation to Brazilian legislation.

Elements	Sampling sites					CONAMA 357. Art. 15	QL
	S1	S2	S3	S4	Ref		
Manganese (Mn)	< 0.01 mg/L	3.23 mg/L*	0.15 mg/L*	24.17 mg/L*	0.05 mg/L	0.1 mg/L	0.01
Iron (Fe)	0.12 mg/L	0.2 mg/L	0.1 mg/L	0.21 mg/L	0.09 mg/L	-	0.01
Aluminum (Al)	0.01 mg/L	0.016 mg/L	< 0.01 mg/L	0.019 mg/L	< 0.01 mg/L	-	0.01
Chromium (Cr)	0.1 mg/L*	0.29 mg/L*	0.09 mg/L*	0.26 mg/L*	0.06 mg/L*	0.05 mg/L	0.01

Copper (Cu)	0.1 mg/L	0.08 mg/L	0.08 mg/L	0.09 mg/L	< 0.001 mg/L	-	0.001
Nickel (Ni)	< 0.01 mg/L	0.025 mg/L	0.01				
Lead (Pb)	1.7 mg/L*	1.9 mg/L*	0.9 mg/L*	0.9 mg/L*	0.2 mg/L*	0.01 mg/L	0.01
Zinc (Zn)	0.8 mg/L	0.87 mg/L*	0.06 mg/L	0.85 mg/L*	0.09 mg/L	0.18 mg/L	0.01
Cadmium (Cd)	< 0.001 mg/L	0.001 mg/L	0.001				
Silicon (Si)	12.414 mg/L	63.370 mg/L	10.021 mg/L	27.530 mg/L	13.269 mg/L	-	0.4

* Indicates values above the legislation (CONAMA 357. Art. 15 for the type I water). (S) sampling sites and (Ref), the reference area. (QL) Quantification limit.

3.2 Bat community

Seventy-six bats of 12 species were collected, all belonging to the Phyllostomidae family, sampled in the Barro Alto and Niquelândia in Goiás, Brazil (Table 2). Of these animals, it was considered only those with at least five individuals sampled. Thus, after screening, 62 animals were used for the biomarkers, comet assay, and the micronucleus test (Fig. 2).

Table 2 Species of bats sampled in the mining area and away from the mining activity.

*Species with at least five individuals used in the analysis.

Family/Subfamily/Species	Sampling points					Trophicguild	Status		
	S1	S2	S3	S4	Ref				
Phyllostomidae									
Carollinae									
<i>Carollia perspicillata</i> *	11	13	12		5	F	LC		
Glossophaginae									
<i>Glossophaga soricina</i> *		5		5		N	LC		
<i>Lonchophylla dekeyseri</i>			1			N	NT		
Desmodontinae									
<i>Desmodus rotundus</i> *		5				H	LC		
Phyllostominae									
<i>Phyllostomus hastatus</i> *			8		O		LC		
<i>Phyllostomus discolor</i>	1								
<i>Lophostoma silvicolum</i>				2					
Stenodermatinae									
<i>Artibeus lituratus</i>		1			F		LC		
<i>Sturnira lilium</i>			1	1	F		LC		
<i>Platyrrhinus lineatus</i>	2				F		LC		

<i>Platyrrhinus incarum</i>	1	F	LC
<i>Artibeus cinereus</i>	1	F	LC

S = Sampling sites. Trophic guild: F = Frugivores, N = Nectarivores, H = Hematophagous and O = Omnivore. LC) Least Concern and NT) Near Threatened. S) sampling sites and Ref) reference área. *Species with at least five individuals used in the analysis.

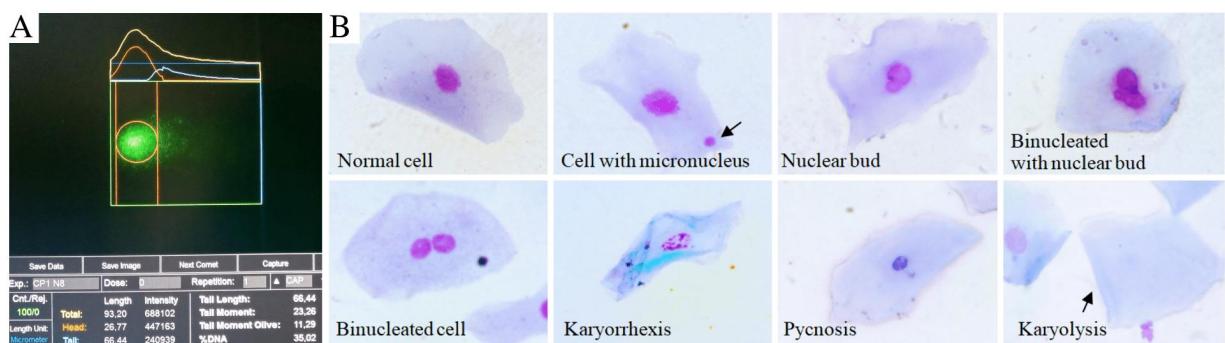


Fig. 2 Photomicrography of biomarkers analyzed in bats. A) A nucleoid being captured during the comet assay analysis and B) Oral mucosal cells highlighting micronuclei and other abnormalities, in addition to evidencing a normal cell (Normal cell) observed during the micronucleus test.

3.3 Comet assay

62 bats of four species were selected for genotoxic analysis (Table 3). At sites 2, 3, and 4, *Glossophaga soricina* and *Carollia perspicillata* showed a higher frequency of DNA damage than *C. perspicillata* at site 1 for almost all comet assay parameters (Table 3). The exception was for *G. soricina* from site 2 in tail length and olive tail moment, which were not different from *C. perspicillata* (S1).

Table 3 DNA damage in bats captured in the mining and reference area from Goiás State, Brazil.

Environment	Species	N	Medium±standard errors of the comet assay parameters		
			%DNA	Taillength	Olive tailmoment
S1	<i>Carollia perspicillata</i>	11	14.03±1.81a	11.87±1.14a	1.81±0.30a
S2	<i>Glossophaga soricina</i>	5	28.41±6.23cd	26.20±4.67ad	5.83±1.30ab
S2	<i>Carollia perspicillata</i>	11	28.32±1.90cd	27.89±2.41d	5.15±0.72b
S2	<i>Desmodus rotundus</i>	5	19.95±5.80ac	28.02±7.13ad	4.07±1.24ab
S3	<i>Carollia perspicillata</i>	12	29.30±2.87cd	28.50±1.97d	6.46±1.20b

S4	<i>Glossophaga soricina</i>	5	32.56±2.48d	32.88±1.87d	6.68±0.60b
S4	<i>Phyllostomus hastatus</i>	8	20.51±2.08ad	12.12±0.84ac	2.52±0.23ab
Ref	<i>Carollia perspicillata</i>	5	15.58±2.93ac	14.49±1.10ad	2.12±0.37ab
Statistics			<i>F</i> = 5.3923; <i>p</i> = 0.0002	<i>H</i> = 30.5608 <i>p</i> < 0.0001	<i>H</i> = 30.6575 <i>p</i> < 0.0001

Different letters indicate a significant difference, while values marked with the same letters are statistically similar. An analysis of variance (ANOVA) followed by the Tukey test or the Kruskal-Wallis H test followed by Dunn's post-hoc were used to compare species at the sampling points. A *p*-value<0.05 was considered for statistical significance. (S): Sampling sites and (Ref): reference area.

3.4 Micronucleus and other nuclear abnormalities test

For the micronucleus test, *C. perspicillata* from site 2 showed a higher average of micronuclei compared to the same species in sites 1 and 3. While at the other sampling points, there was no significant difference (Table 4). Considering other abnormalities, a higher frequency of nuclear bud was found in *C. perspicillata* in S2. *Phyllostomus hastatus* was the species that presented the highest frequency of binucleate (S4), as well as *C. perspicillata* for karyolysis (S3), both animals from the mining area (Table 4).

Table 4 Mean frequency of micronucleus and other nuclear abnormalities in bats captured in the mining and reference area from Goiás state, Brazil.

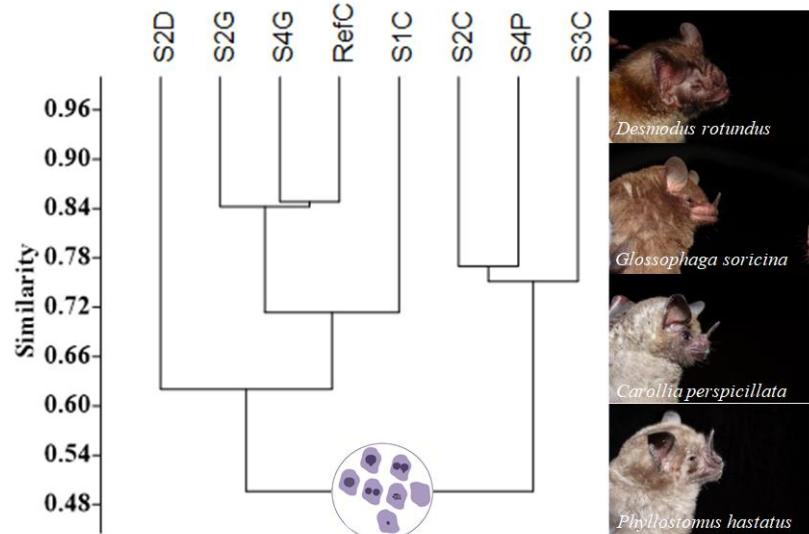
Species	Env	Mean ± standard error					
		MN	NB	BI	PY	KX	KY
<i>C. perspicillata</i>	S1	0.64±0.28a	0.75±0.33ab	1.82±0.42ab	2.91±0.98a	0.45±0.25a	2.27±0.56a
<i>G. soricina</i>	S2	0.60±0.24ab	0.60±0.40a	1.60±0.51ab	3.20±1.59a	1.40±0.75a	6.60±1.42ab
<i>C. perspicillata</i>	S2	1.91±0.39b	2.45±0.62b	1.45±0.31ab	6.73±1.88a	2.64±0.93a	10.27±0.83ab
<i>D. rotundus</i>	S2	1.20±0.58ab	1.20±0.58ab	1.00±0.32ab	2.80±1.46a	1.00±0.77a	18.00±1.72ab
<i>C. perspicillata</i>	S3	0.42±0.20a	0.17±0.11a	4.67±1.06ab	7.42±2.15a	0.08±0.08a	11.75±0.69b
<i>G. soricina</i>	S4	0.40±0.24ab	0.60±0.40ab	2.00±0.32a	4.20±1.11a	3.60±1.75a	7.80±1.00ab
<i>P. hastatus</i>	S4	1.13±0.35ab	1.25±0.37ab	4.38±0.71b	9.13±2.32a	0.63±0.26a	8.75±1.01ab
<i>C. perspicillata</i>	Ref	0.40±0.24ab	1.00±0.55ab	0.40±0.24a	4.20±1.91a	1.00±0.63a	8.20±1.00ab
Statistic		<i>F</i> = 3.0056; <i>p</i> = 0.0098	<i>F</i> = 3.2107; <i>p</i> = 0.0066	<i>H</i> = 21.957; <i>p</i> = 0.0026	<i>F</i> = 1.5418; <i>p</i> = 0.1727	<i>H</i> = 10.3178; <i>p</i> = 0.171	<i>H</i> = 16.6427; <i>p</i> = 0.019

Different letters indicate a significant difference, while values marked with the same letters are statistically similar. An analysis of variance (ANOVA) followed by the Tukey test or the Kruskal-Wallis H test followed by Dunn's post-hoc were used to compare species at the sampling points. A *p*-value<0.05 was considered for statistical significance. MN) Micronuclei,

NB = Nuclear Bud, BI = Binucleated, PY = Pyknosis, KX = Karyorrhexis, and KY = Karyolysis. Env = Environment, S = Sampling sites and Ref = Reference area

A similarity clustering dendrogram was performed for the ANs (total nuclear abnormalities) concerning the species at the sampling sites (Fig. 3). Two groups of species according to the similarity in the proportion of ANs were observed, being formed by *Carollia perspicillata* (S2 and S3) and *Phyllostomus hastatus* (S4). In contrast, the second group was formed by *Carollia perspicillata* (S1, S2, and Reference Area) with *Glossophaga soricina* (S4). Although *Desmodus rotundus* can be considered the S2 group, it was similarly somewhat distant.

Fig. 3 Dendrogram of similarity (Bray-Curtis Cluster Analysis) between the sampling points and the total number of NAs. S = Sampling sites followed by the species initials



3.5 Females and males

C. perspicillata was the most common species among the sampling sites. Then, comparing the sex of environmentally exposed animals, no difference was observed between female and male of *C. perspicillata* in the mining area (Table 5). In this analysis, it was not considered the population of the reference area because the population of males and females was less than five individuals.

Table 5 Comparison of genotoxic and mutagenic damages between *C. perspicillata* females and males in the open pit area.

Variables	Mean±standard error		<i>t</i> -value	<i>p</i> -value
	Female (=20)	Male (n=14)		

%DNA	22.28±1.98	26.56±3.11	-1.22001	0.2313
Taillength	20.32±2.39	26.65±4.07	-1.42538	0.1637
Olive tailmoment	3.89±0.63	5.46±1.14	-1.29606	0.2042
Micronucleusfrequency	1.00±0.28	0.93±0.29	0.17299	0.8637
Total NAs	20.05±2.57	16.93±3.71	0.71648	0.4788

NAs: Nuclear abnormalities

3.6 Genotoxic and mutagenic particularity at sampling sites

In some sites, more than one species was captured, as shown in Figure 4. At site 2, *G. soricina*, *C. perspicillata*, and *D. rotundus* do not differ in the DNA damage frequency (Fig. 4A). At site 4, the nectarivore *G. soricina* showed more significant DNA damage than the large omnivore *P. hastatus* (Fig. 4B). There was no difference between the species within the sampling sites (Fig. 4C and D).

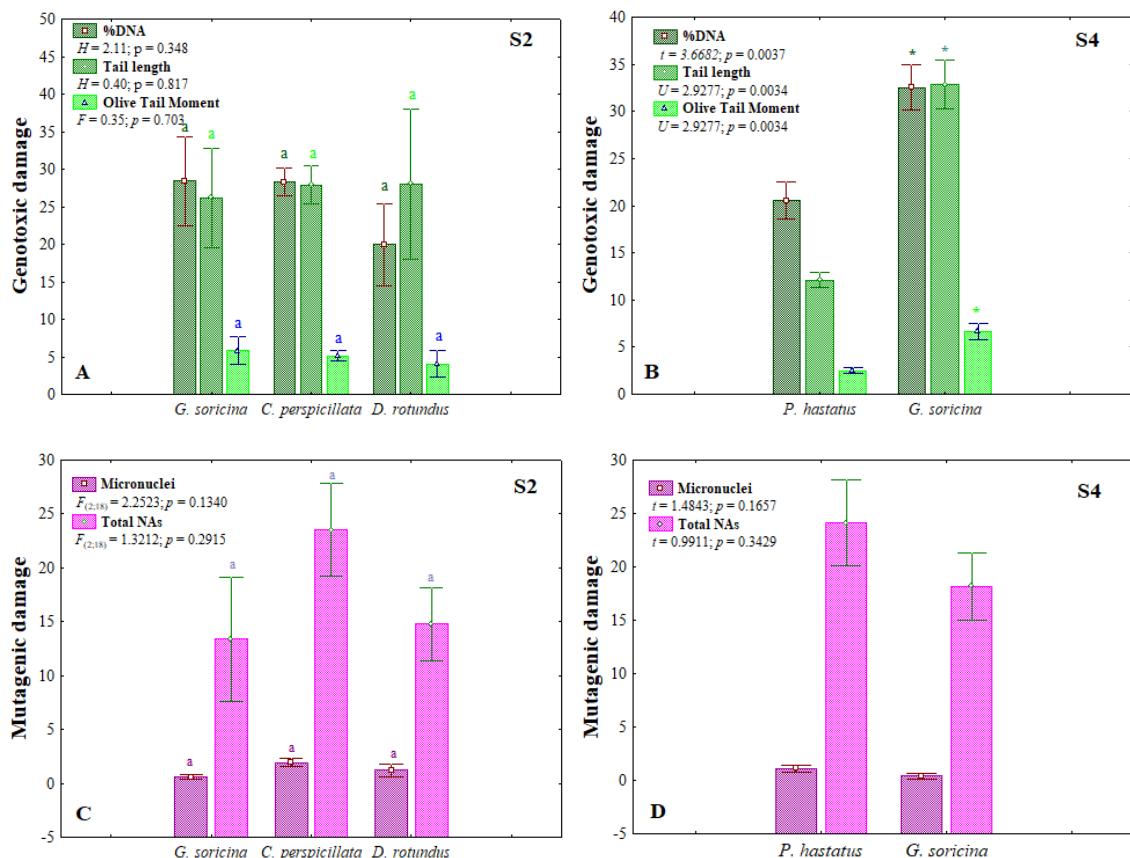


Fig. 4 DNA damage between bats at the same sampling point. Different letters indicate a significant difference, while values marked with the same letters are statistically similar. At the end of the bars, it indicates the mean and the variation of the standard error. A $p < 0.05$ was considered for statistical significance. Tail length (TL), the DNA percentage in tail (% DNA), and Olive's Tail Moment (MCO)

4. Discussion

To be analyzed for genotoxic and mutagenic damages, it was evaluated chiropterans of four species (*C. perspicillata*, *G. soricina*, *P. hastatus*, and *D. rotundus*) exposed to iron-nickel mining activity. In addition, for cytotoxic damage, frequencies of karyorrhexis, pyknosis and karyolysis were also recorded. This is the first study with the hematophagous species *D. rotundus* and the omnivore *P. hastatus* using comet assay and micronucleus test for sensitivity to the effect of open-pit mining. It was observed that populations generally responded differently in the frequency of DNA damage across the mining area. The environments are classified as sampling sites, such as S1, which, although it is in the ore extraction plant, the species *C. perspicillata* indicated minor genotoxic damage. In this site, the animals were obtained in an area of a very dense riparian forest that had a direct influence on the ore extraction, but possibly the species used the area as a transition corridor.

The reference area, as expected, was distant from the ore extraction area, and the animals (*C. perspicillata*) also showed low DNA damage. Thus, it was found that at site 2, the animals (*G. soricina*, *C. perspicillata*, and *D. rotundus*) were in the largest spatial radius of exposure to the mining company (26.4% of the mining area considering a radius of 2 km from the collection point), and DNA lesions were high for *C. perspicillata* (81.77%) and *G. soricina* (82.35%) about the reference area, 102 and 101% compared to *C. perspicillata* from S1. At site three, *C. perspicillata* and *G. soricina*, where the radius of mining activities along the capture point was 10.78 and 18.4%, respectively, had an 88.6 and 109% increase in genotoxic damage related to the reference area.

This leads us to assume that these two species are sensitive, regardless of the size of the mining area close to their capture and foraging sites. This comparison is still a bit complex, as it only considers the area used for ore exploration that we have determined in the buffer and does not consider, for example, possible levels of atmospheric pollution in the area, whether dust particles or smoke from heavy vehicles (carbon monoxide, nitrogen oxides, hydrocarbons, sulfur oxides, and particulate matter) or places of shelter for the species. Sunset air pollution from industrial activities, namely dust particles and chemical pollutants, is recognized to harm the health and survival of bats (Voigt et al. 2018).

In addition, sites 1 and 2 were distant just over 100 meters (in a straight line) from the ore extraction area. Study in the area, inside, and close to the foundry; dust ingestion rates can be higher than 50 mg/day, posing a risk to humans if workers do not have safety measures such as masks/filters (Ettler et al. 2018). However, this is possibly an imminent inhalation hazard

for aerial fauna such as bats.

Site 4 covered the mining company's smelting area among the sampling areas. While sites 1 and 2 were approximately 3 km away. For the micronucleus test of bat community in the different points, *C. perspicillata* (S2) was the only species that presented a higher frequency of micronuclei (198%) than *C. perspicillata* S1, corroborating the comet assay, and also had 355% more micronucleus compared to S3 (*C. perspicillata*). For other nuclear abnormalities, the nuclear buds resulting from gene amplification (Ernst et al., 2021), were as expressive in *C. perspicillata* at S2 as the micronucleus frequency. According to Bolognesi et al. (2013) these are biomarkers of DNA damage. Binucleated cells that reflect mitotic disorders and failure in cytokinesis (Ernst et al. 2021) were more expressive for *Phyllostomus hastatus*. While the markers that reflect cytotoxic effects were higher for karyolysis in *C. perspicillata* (S3) from the mining area.

Other cytotoxic markers such as karyorrhetic and pyknotic cells, cell death reflexes (Bolognesi et al. 2013) showed no difference within the bat community. On the other hand, the sum of these abnormalities indicated greater similarity between populations of nectarivores in the mining area, as well as between nectarivores (mining) and frugivores in the reference area. Oral epithelial cells form the first barrier to the digestive and respiratory tract, so the changes observed in oral epithelial cells may provide evidence of possible side effects from exposure to harmful agents (Thanasias et al. 2019), such as potentially harmful metals toxics in the mining area.

Long-term exposure to metals typically occurs through the air, water, soil, and food. Considering a dermal exposure, bats have many blood vessels in the wing membrane (Mina et al. 2019), and when considering their relatively large home range for some species, they are susceptible to contact with potentially toxic dust particles (Voigt et al. 2018) either on fur or direct skin contact. A study evaluating the bioaccumulation between the hair and the wing membrane indicated high concentrations of Cr, Cu, Mn, Ni, Pb, Se, and Zn in the wing of Vespertilionidae bats from wind farms, showing a particular probability of accumulating some metals in the wings, more than fur (Mina et al. 2019). Thus, the toxic particles in the hair reach the skin and are absorbed, and therefore it is possible to verify and quantify DNA damage.

There was no difference in genotoxic and mutagenic damage in comparing *C. perspicillata* males and females. In studies with small mammals, *Biomys musculus* exposed to mining residues indicated zinc, nickel, iron, and manganese (Tovar-Sánchez et al. 2012). In that study, the authors observed that females had significantly higher DNA damage levels than males, regardless of the study site (control vs. exposed). It is essential to understand the

relationship between sex and genotoxic damage, as metals such as nickel may be associated with oxidative stress in the reproductive system (Rizvi et al. 2020), although it was not found this element in the studied area. Oxidative stress influences zinc metabolism, which is critical for sperm stability, and affects the structure of DNA-binding proteins, including protamines, thus affecting the function of these germ cells (Rizvi et al. 2020). Nevertheless, in situ studies, in which multiple metals and trace elements are usually present, the impact on the body can be more complex.

Considering the particularity of sites where more than one species was captured, there is no difference in the biomarkers in the species *G. soricina*, *C. perspicillata*, and *D. rotundus* in site 2. Nevertheless, in site 4, *G. soricina* and *P. hastatus* presented genotoxic differences (in the comet assay parameters). The sensitivity of DNA lesion development in *G. soricina* was observed for all parameters of the comet assay. Thus, the present study agrees with Sotero (2022), who also observed that the nectarivore *G. soricina* exposed to the mining area is sensitive to the development of DNA damage, whose attributes such as faster metabolism due to diet, may be a plausible explanation for the DNA damage of such species. Typically, baseline levels of DNA damage vary depending on the studied species and cell type, making it necessary to determine the optimal conditions for both tests (Zapata et al. 2016). However, the present study strengthens the importance of analyzing blood and exfoliated cells from the oral mucosa in animals exposed to mining activity.

For other organisms, such as humans, an analysis of exfoliated cells of the oral mucosa in occupational workers at a ferronickel factory indicated a higher frequency of DNA damage compared to the control group (Letaj et al. 2014). For birds such as wild pigeons (*Columba livia*) that live near ferronickel smelters, Milaimi et al. (2016) observed that Ni concentrations were high in tissues such as the liver, kidney, testes, femur, and tibia, whose highest concentration was recorded in the liver (139.97 µg/mg d.w.). While in aquatic environments, nickel has genotoxic potential in multiple organisms, such as fishes (Singh et al. 2019). Assuming that nickel compounds can interfere with DNA repair mechanisms, it also potentiates the increase in the effects of different genotoxic agents (Annangi et al. 2016), which can be a problem when considering many environmental stressors.

Four metals were above the quantification limit of Brazilian legislation, namely Mn, Cr, Pb, and Zn. In addition to these elements above the recommended, others such as Fe, Al, Cu, and Si do not have recommendations in Brazil for type I freshwater. According to Kocadal et al. (2020), heavy metals can induce genetic damage through multiple pathways, such as micronucleus formation, chromosomal aberrations, other anomalies, and DNA damage at the

molecular level. Although we have not quantified it in bat tissue, analyses of the region's water indicated that many elements are at high levels in the environment and the animals are susceptible to bioaccumulation through water or food consumption, mainly aquatic insects, in this case, considering biomagnification.

Mn is a necessary micronutrient for the biological aspect. However, when found over cellular levels, Mn is harmful (Balachandran et al. 2020). For Cr, the primary mechanism of DNA damage is the generation of oxidative stress through the production of reactive intermediates that can disrupt cellular integrity and functions, compromising DNA, membrane proteins, and lipids (Kocadal et al. 2020). Pb is also a versatile, subtle, and persistent poison (Danadevi et al. 2003). It can replace calcium and zinc in enzymes involved in DNA processing and repair, leading to an inhibition of DNA repair and an increase in genotoxicity when combined with other DNA-damaging agents (García-Leston et al. 2010). In addition, the oxidative stress produced by increased levels of free radicals induced by exposure to lead may also contribute to the indirect genotoxicity of this metal (García-Leston et al. 2010). Although Zn is necessary for the biological system, a prolonged intake with high levels can be toxic to the body. Given the context, the genotoxicity and mutagenicity observed in bats in the mining area are evident.

Metals still contaminate a wide range of habitats, but the risks to bats remain poorly understood (Hernout et al. 2016). In the present study, insectivorous species were not obtained. Perhaps this is one of the groups of bats most affected in the ferronickel mining area because they are exclusive predators of insects, occupying the highest trophic level. In addition to exposing trace elements through ingestion of insects, they can consume contaminated water and have dermal exposure and inhalation (Andreani et al. 2019). Some of the species in the present study have already been reported in other investigations involving mining, such as artisanal gold exploration, citing mercury bioaccumulation in *G. soricina* and *C. perspicillata* in Peru (Carrasco-Rueda et al. 2020). *D. rotundus* and *G. soricina* were also associated with mercury bioaccumulation related to diet connectivity to aquatic environments (Becker et al. 2018), which shows the susceptibility of these animals in ore extraction areas, whose stressors may be capable of to compromise the sustainability of the species.

5. Conclusion

In a ferronickel mining area, we provided data on cytotoxic, genotoxic and mutagenic damage in *C. perspicillata*, *G. soricina*, *P. hastatus* and *D. rotundus*. DNA damage varied between bat species and populations, possibly reflecting exposure to metals. Metals such as

Mn, Cr, Pb, and Zn at high levels can be toxic in the region for animals. We also do not exclude other types of pollutants such as carbon monoxide, nitrogen oxides, hydrocarbons, sulfur oxides, and particulate matter generated by machinery and automobiles in the mining area. Overall, genotoxicity is an important toxicological outcome due to the link with disease and therefore needs to be better understand in bats. Future studies can explore the impact of mining at the gene and protein level.

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7. Ethical Approval The present study was approved by the Ethics Committee on the Use of Animals of the Federal University of Goiás (n.30/21) and by the Chico Mendes Institute for Biodiversity Conservation (n. 69513-2).

8. Consent to Participate NA

9. Consent to Publish The researchers of the present study are in agreement with the publication.

10. Authors Contributions M. B. S Methodology, formal analysis, investigation, writing-original draft, visualization, validation. D.F.S Methodology, visualization, validation. C.G.A.S., R.A.A and R. E. B Validation, visualization. L.R.S.S and D.M.S Project administration, supervision, writing-review & editing, visualization, validation.

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13. Availability of data and materials All data obtained in the research are already contained in this manuscript.

14. References

- Andreani G, Cannavaccioulo A, Menotta S, Spallucci V, Fedrizzi G, Carpene E, Isani G (2019) Environmental exposure to non-essential trace elements in two bat species from urbanised (*Tadaridateniotis*) and open land (*Miniopterusschreibersii*) areas in Italy. Environ Pollut 254: 113034. <https://doi.org/10.1016/j.envpol.2019.113034>
- Annangi B, Bonassi S, Marcos R, Hernández A (2016) Biomonitoring of humans exposed to arsenic, chromium, nickel, vanadium, and complex mixtures of metals by using the micronucleus test in lymphocytes. Mutat Res - Rev Mutat Res 770:140-161. <https://doi.org/10.1016/j.mrrev.2016.03.003>
- Balachandran RC, Mukhopadhyay S, McBride D, Veevers J, Harrison FE, Aschner M, Haynes EN, Bowman AB (2020) Brain manganese and the balance between essential roles and neurotoxicity. J Biol Chem 295:6312-6329. <https://doi.org/10.1074/jbc.REV119.009453>
- Becker DJ, Chumchal MM, Broders HG, Korstian JM, Clare EL, Rainwater TR, Platt SG, Simmons NB, Fenton MB (2018) Mercury bioaccumulation in bats reflects dietary connectivity to aquatic food webs. Environ Pollut 233:1076-1085. <https://doi.org/10.1016/j.envpol.2017.10.010>
- Benvindo-Souza M, Borges RE, Pacheco SM, de Souza Santos LR (2019a) Genotoxicological analyses of insectivorous bats (Mammalia: Chiroptera) in central Brazil: The oral epithelium as an indicator of environmental quality. Environ Pollut 245:504-509. <https://doi.org/10.1016/j.envpol.2018.11.015>
- Benvindo-Souza M, Borges RE, Pacheco SM, de Souza Santos LR (2019b) Micronucleus and other nuclear abnormalities in exfoliated cells of buccal mucosa of bats at different trophic levels. Ecotoxicol Environ Saf 172:120-127. <https://doi.org/10.1016/j.ecoenv.2019.01.051>
- Benvindo-Souza M, Hosokawa AV, Dos Santos CGA, de Assis RA, Pedroso TA, Borges RE, Pacheco SM, Souza Santos LR, Silva DDM (2022) Evaluation of genotoxicity in bat species found on agricultural landscapes of the Cerrado savanna, central Brazil. Environ Pollut 118579. <https://doi.org/10.1016/j.envpol.2021.118579>
- Buxton S, Garman E, Heim KE, Lyons-Darden T, Schlekat CE, Taylor MD, Oller AR (2019) Concise review of nickel human health toxicology and ecotoxicology. Inorganics 7:89. <https://doi.org/10.3390/inorganics7070089>
- Calao-Ramos C, Gaviria-Angulo D, Marrugo-Negrete J, Calderon-Rangel A, Guzman-Teran C, Martínez-Bravo C, Mattar S (2021) Bats are an excellent sentinel model for the detection of genotoxic agents. Study in a Colombian Caribbean region. Acta Trop 106141.

- <https://doi.org/10.1016/j.actatropica.2021.106141>
- Carrasco-Rueda F, Loiselle BA, Frederick PC (2020) Mercury bioaccumulation in tropical bats from a region of active artisanal and small-scale gold mining. *Ecotoxicology* 29(7):1032-1042. <https://doi.org/10.1007/s10646-020-02195-3>
- Danadevi K, Rozati R, Banu BS, Rao PH, Grover P (2003) DNA damage in workers exposed to lead using comet assay. *Toxicology* 187:183-193. [https://doi.org/10.1016/S0300-483X\(03\)00054-4](https://doi.org/10.1016/S0300-483X(03)00054-4)
- dos Santos Pedroso-Fidelis G, Farias HR, Mastella GA, Boufleur-Niekraszewicz LA, Dias JF, Alves MC, Silveira PCL, Nesi RT, Carvalho F, Zocche JJ, Pinho RA (2020) Pulmonary oxidative stress in wild bats exposed to coal dust: A model to evaluate the impact of coal mining on health. *Ecotoxicol Environ Saf* 191:110211
- El-Habit H, Moneim AEA (2014) Testing the genotoxicity, cytotoxicity, and oxidative stress of cadmium and nickel and their additive effect in male mice. *Biol Trace Elem Res* 159:364-372. <https://doi.org/10.1007/s12011-014-0016-6>
- Ettler V, Polák L, Mihaljevič M, Ratié G, Garnier J, Quantin C (2018) Oral bioaccessibility of inorganic contaminants in waste dusts generated by laterite Ni ore smelting. *Environ. Geochem. Health* 40:1699-1712. <https://doi.org/10.1007/s10653-016-9875-4>
- Elshkaki A, Reck BK, Graedel TE (2017) Anthropogenic nickel supply, demand, and associated energy and water use. *Resour Conserv Recycl* 125:300-307. <https://doi.org/10.1016/j.resconrec.2017.07.002>
- Ferrante M, Pappalardo AM, Ferrito V, Pulvirenti V, Fruciano C, Grasso A, Sciacca S, Tiagano C, Copat C (2017) Bioaccumulation of metals and biomarkers of environmental stress in *Parablennius sanguinolentus* (Pallas, 1814) sampled along the Italian coast. *Mar Pollut Bull* 122:288-296. <https://doi.org/10.1016/j.marpolbul.2017.06.060>
- Ferrer A (2003) Intoxicación por metales. Metal poisoning. *An Sist Sanit Navar* 26:141–153. <https://scielo.isciii.es/pdf/asisna/v26s1/ocho.pdf>
- Flache L, Ekschmitt K, Kierdorf U, Czarnecki S, Düring RA, Encarnaçao JA (2016) Reduction of metal exposure of Daubenton's bats (*Myotis daubentonii*) following remediation of pond sediment as evidenced by metal concentrations in hair. *Sci Total Environ* 547:182-189. <https://doi.org/10.1016/j.scitotenv.2015.12.131>
- García-Leston J, Méndez J, Pásaro E, Laffon B (2010) Genotoxic effects of lead: an updated review. *Environ Int* 36:623-636. <https://doi.org/10.1016/j.envint.2010.04.011>
- Gallant LR, Grooms C, Kimpe LE, Smol JP, Bogdanowicz W, Stewart RS, Clare EL, Elizabeth L, Fenton MB, Blais JM (2020) A bat guano deposit in Jamaica

- recorded agricultural changes and metal exposure over the last > 4300 years. *Palaeogeogr. Palaeoclimatol Palaeoecol* 538:109470. <https://doi.org/10.1016/j.palaeo.2019.109470>
- Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S., Catalano, A. (2020). Nickel: Human health and environmental toxicology. *International journal of environmental research and public health*, 17(3), 679. <https://doi.org/10.3390/ijerph17030679>
- Gonçalves MW, de Oliveira HHP, Sousa CCN, Carvalho WF, Nomura F, Maciel NM, Bastos RP (2012) Análises mutagênicas de anuros em áreas de mineração de níquel. *Revista EVS-Revista de Ciências Ambientais e Saúde* 37:737-747. <http://dx.doi.org/10.18224/est.v39i2.2592>
- Gonçalves VF (2022) Anomalias nucleares eritrocitárias como ferramentas de biomonitoramento por aves no cerrado. Universidade Federal de Uberlândia. Tese - Ecologia e Conservação de Recursos Naturais. <http://orcid.org/0000-0002-5181-4165>
- Hernout BV, Arnold KE, McClean CJ, Walls M, Baxter M, Boxall AB (2016) A national level assessment of metal contamination in bats. *Environ Pollut* 214:847-858. <https://doi.org/10.1016/j.envpol.2016.04.079>
- Kocadal K, Alkas FB, Battal D, Saygi S (2020) Cellular pathologies and genotoxic effects arising secondary to heavy metal exposure: a review. *Hum Exp Toxicol* 39:3-13. <https://doi.org/10.1177/0960327119874439>
- Kozlowski H, Kolkowska P, Watly J, Krzywoszynska K, Potocki S (2014) General aspects of metal toxicity. *Curr Med Chem* 21:3721-3740.
- Lagunas-Rangel FA (2020) Why do bats live so long?—Possible molecular mechanisms. *Biogerontology* 21:1-11. <https://doi.org/10.1007/s10522-019-09840-3>
- Letaj KR, Kurteshi KH (2014) Genotoxic and cytotoxic effect of nickel in buccal cell at workers in ferronickel factory. *Regional development of Central and Eastern European countries*, 287
- Mina R, Alves J, da Silva AA, Natal-da-Luz T, Cabral JA, Barros P, Topping CJ, Sousa JP (2019) Wing membrane and fur samples as reliable biological matrices to measure bioaccumulation of metals and metalloids in bats. *Environ Pollut* 253:199-206. <https://doi.org/10.1016/j.envpol.2019.06.123>.
- Milaimi AP, Selimi Q, Letaj K, Trebicka A, Milaimi A (2016) Accumulation of Heavy Metals in Feral Pigeons Living Near a Ferronickel Smelter. *Pol. J Environ Stud* 25. <https://doi.org/10.15244/pjoes/63425>.
- Podlutsky AJ, Khritankov AM, Ovodov ND, Austad SN (2005) A new field record for bat longevity. *J Gerontol A* 60:1366–1368. <https://doi.org/10.1093/gerona/60.11.1366>.

- Reis NR, Fregonezi MN, Peracchi AL, Shibatta OA (2013) Morcegos do Brasil: Guia de Campo. Rio de Janeiro: Technical Books. Londrina
- Rizvi A, Parveen S, Khan S, Naseem I (2020) Nickel toxicology with reference to male molecular reproductive physiology. *Reprod Biol* 20:3-8. <https://doi.org/10.1016/j.repbio.2019.11.005>.
- Silva IHF, Braga FCS (2020) Análise dos depósitos de lateritas niquelíferas do Brasil a partir do conceito de sistemas minerais holísticos. *Estudos Geológicos (UFPE)*, 30:79-99. <https://doi.org/10.18190/1980-8208/estudosgeologicos.v30n1p79-99>
- Singh M, Khan H, Verma Y, Rana SVS (2019) Distinctive fingerprints of genotoxicity induced by As, Cr, Cd, and Ni in a freshwater fish. *Environ Sci Pollut Res* 26:19445-19452. <https://doi.org/10.1007/s11356-019-05274-z>
- Singh S, Kumar V, Singh P, Banerjee BD, Rautela RS, Grover SS, Rawat DS, Pasha ST, Jain SK, Rai A (2012) Influence of CYP2C9, GSTM1, GSTT1 and NAT2 genetic polymorphisms on DNA damage in workers occupationally exposed to organophosphate pesticides. *Mutat Res* 741:101-108. <https://doi.org/10.1016/j.mrgentox.2011.11.001>
- Sotero DF (2022) Análises genotóxica e mutagênica de espécies de morcegos de uma mineradora do Estado de Goiás. Dissertação do curso de Pós-Graduação em Genética e Biologia Molecular. Universidade Federal de Goiás
- Tognacchini A, Rosenkranz T, van der Ent A, Machinet GE, Echevarria G, Puschenreiter M (2020) Nickel phytomining from industrial wastes: Growing nickel hyperaccumulator plants on galvanic sludges. *J Environ Manage* 254:109798. <https://doi.org/10.1016/j.jenvman.2019.109798>
- Tovar-Sánchez E, Cervantes LT, Martínez C, Rojas E, Valverde M, Ortiz-Hernández ML, Mussali-Galante P (2012) Comparison of two wild rodent species as sentinels of environmental contamination by mine tailings. *Environ Sci Pollut Res* 19:1677-1686. <https://doi.org/10.1007/s11356-011-0680-4>
- Thanasis E, Koutsoumplias D, Vlastos D, Halkos G, Matthopoulos D, Makropoulos V (2019) Evaluation of Genetic Damage to Workers in a Nickel Smelting Industry. *OccupEnviron Med* 7:21. <https://doi.org/10.4236/odem.2019.71003>
- Strauch JCM, Valente K, Ajara Cesar, Teixeira MP, Cardoso SC (2011) Grandes Mineradoras e a comunidade em Niquelandia (GO). In: Francisco Rego Chaves Fernandes, Maria Amélia Rodrigues da Silva Enriíquez, Renata de Carvalho Jimenez Almino. (Org.). Recursos Minerais & Sustentabilidade Territorial: Grandes Minas. 1ed. Rio de Janeiro: CETEM/MCT, 2:135-162

Voigt CC, Rehnig K, Lindecke O, Pētersons G (2018) Migratory bats are attracted by red light but not by warm-white light: Implications for the protection of nocturnal migrants. *Ecol Evol* 8:9353-9361. <https://doi.org/10.1002/ece3.4400>

Zapata, L.M., Bock, B.C., Orozco, L.Y., Palacio, J.A., 2016. Application of the micronucleus test and comet assay in *Trachemys callirostris* erythrocytes as a model for in situ genotoxic monitoring. *Ecotoxicol. Environ. Saf.* 127, 108-116.
<https://doi.org/10.1016/j.ecoenv.2016.01.016>

Zukal J, Pikula J, Bandouchova, H (2015) Bats as bioindicators of heavy metal pollution: history and prospect. *MammBiol* 80:220–227.
<https://doi.org/10.1016/j.mambio.2015.01.001>

CONCLUSÃO GERAL

Os morcegos possuem sensíveis a poluentes e com isso são considerados bons bioindicadores da saúde ambiental. Uma vez detectadas substâncias presentes no corpo dos animais ou danos, como, por exemplo, fisiológicos e/ou genéticos é possível determinar o estado de saúde dos animais e dos ambientes onde eles visitaram. Nesse contexto, (**Capítulo 1**) na busca pela compreensão sobre os estudos no campo da ecotoxicologia com morcegos no Brasil, foi constatado que existe uma escassez de pesquisas sobre esse tópico. Somente nove espécies de morcegos já foram estudadas, sendo *Artibeus lituratus* a espécie mais representada. Xenobióticos como metais pesados e pesticidas foram os principais interesses dos pesquisadores e os impactos em morcegos ocorreram a nível celular, metabolismo e na reprodução. O baixo número de estudos pode ser um indicativo da falta de pesquisadores que investigam essa temática no Brasil.

Nesse sentido, biomarcadores pouco invasivos e de baixo custo como o teste de micronúcleo em células esfoliadas de mucosa bucal (para rastrear dano mutagênico), bem como o ensaio cometa (dano genotóxico) utilizando sangue periférico indicaram boas alternativas para cientistas da conservação de morcegos. Esses biomarcadores (**Capítulo 2**) responderam precisamente a detecção de danos no DNA em morcegos de áreas de lavouras de cana-de-açúcar e soja em relação àqueles de áreas conservadas. No nosso estudo, *C. perspicillata* foi uma espécie comum nos três ambientes investigados. O dano ao DNA registrado nesta espécie foi significativamente mais elevado na área de cana-de-açúcar em todos os parâmetros do ensaio cometa em relação à área de controle.

Danos genotóxicos e mutagênico também foram detectados nas amostras de nectarívoros *G. soricina* e insetívoros *M. molossus* da área de soja em relação a mesma espécie da área controle. *G. soricina* e *M. albescens* tiveram peso corpóreo maior na unidade de conservação em relação à área agrícola. No entanto, nenhuma correlação significativa foi encontrada entre o peso corporal e quaisquer parâmetros genotóxicos entre essas espécies. Embora, não tenhamos conseguido isolar xenobióticos alvos para tais danos observados, a paisagem antropizada onde os animais foram capturados indicava claramente baixa qualidade de vegetação nativa comparada à unidade de conservação, o que indica maiores chances de exposição dos animais nas áreas de cultivo.

No **Capítulo 3** demonstramos que indivíduos da espécie *G. soricina* amostrados em área de mina apresentaram maior frequência de dano no DNA em relação a *C. perspicillata* da área de referência. Por outro lado, não observamos diferença entre as espécies em relação ao

dano mutagênico (micronúcleos). Houve diferença entre as espécies dentro da área de mineração. O estudo não detectou diferença entre a relação machos e fêmeas, mas isso possivelmente ao baixo número de espécies, com excessão de *C. perspicillata* a única investigada. Esse estudo também chama atenção para elementos como Mn, Cr, Pb e Zn observados em amostras de água estavam em níveis elevados na área de mineração. O que pode ser um risco para biocumulação na biodiversidade local.

Supplementary Material 1 (Capítulo 2)

Table 1. Bats and percentage of native vegetation at sampling sites.

Environment and Species	Animals by sites and percentage of native vegetation					Abundance
	S1	S2	S3	S4	S5	
ENP	95.2%	100%	100%	97.7%	99.7%	33
Phyllostomidae						
<i>Glossophaga soricina</i>	7	1	1	2	1	12
<i>Carollia perspicillata</i>	8	1	0	0	0	9
Molossidae						
<i>Molossus molossus</i>	5	0	0	1	0	6
Vespertilionidae						
<i>Myotis albescens</i>	5	0	0	0	1	6
Sugarcane	41.68%	20.6%	11.34%	5.98%	10.01%	35
Phyllostomidae						
<i>Artibeus lituratus</i>	6	0	0	0	0	6
<i>Carollia perspicillata</i>	0	1	8	2	1	12
<i>Phyllostomus hastatus</i>	3	0	0	9	0	12
Vespertilionidae						
<i>Myotis albescens</i>	4	0	1	0	0	5
Soybean	20.37%	22.26%	15.04%	19.61%	18.94%	58
Phyllostomidae						
<i>Artibeus lituratus</i>	0	0	2	5	0	7
<i>Glossophaga soricina</i>	3	0	9	0	0	12
<i>Carollia perspicillata</i>	5	0	6	0	0	11
<i>Platyrrhinus lineatus</i>	0	0	0	7	0	9
<i>Artibeus planirostris</i>	3	0	2	6	1	12
Molossidae						
<i>Molossus molossus</i>	0	5	2	0	0	7
Total Abundance						126
Species Richness						8

S = sampling site. The number in front of the "S" reports the sampling site. Emas National Park (ENP).

Supplementary Material 2 (Capítulo 2)

Table 2. Physical-chemical analysis of water at sampling points.

ENP	S1	S2	S3	S4	S5	LQ
Glyphosate+Ampa	<105,00	<105,00	<105,00	<105,00	<105,00	105,00
2,4D+2,4,5T	<1,15	<1,15	<1,15	<1,15	<1,15	1,15
Alaclor	<0,100	<0,100	<0,100	<0,100	<0,100	0,100
Aldicarb + Aldicarbsulfone+aldicarbsulfoxide	<10	<10	<10	<10	<10	10
Aldrin+Dieldrin	<0,00200	<0,00200	<0,00200	<0,00200	<0,00200	0,00200
Atrazine	<1,00	<1,00	<1,00	<1,00	<1,00	1,00
Carbendazine+Benomil	<20,000	<20,000	<20,000	<20,000	<20,000	20.000
Carbofuran	<5,000	<5,000	<5,000	<5,000	<5,000	5.000
ChlordanCis + Trans	<0,020	<0,020	<0,020	<0,020	<0,020	0,020
Chlorpyrifos+ChlorpyrifosOxon	<5,100	<5,100	<5,100	<5,100	<5,100	5.100
Diuron	<50,000	<50,000	<50,000	<50,000	<50,000	50.000
EndosulfanAlpha+EndosulfanBeta+Endosulfan						
Sulfate	<0,030	<0,030	<0,030	<0,030	<0,030	0,030
Endrin	<0,001000	<0,001000	<0,001000	<0,001000	<0,001000	0,001000
Gamma-BHC (Lindano)	<0,010	<0,010	<0,010	<0,010	<0,010	0,010
Methamidophos	<5,000	<5,000	<5,000	<5,000	<5,000	5.0000
Molinate	<0,100	<0,100	<0,100	<0,100	<0,100	0,100
o,p-DDD+ o,p-DDE+ o,p-DDT	<0,0030	<0,0030	<0,0030	<0,0030	<0,0030	0,0030
MethylParathion	<0,050	<0,050	<0,050	<0,050	<0,050	0,050
Pendimethalin	<0,100	<0,100	<0,100	<0,100	<0,100	0,100
Permethrin	<0,200	<0,200	<0,200	<0,200	<0,200	0,200
Profenophos	<0,100	<0,100	<0,100	<0,100	<0,100	0,100
Simazine	<0,100	<0,100	<0,100	<0,100	<0,100	0,100
Tebuconazole	<0,100	<0,100	<0,100	<0,100	<0,100	0,100
Terbuphos	<0,100	<0,100	<0,100	<0,100	<0,100	0,100
Trifluralin	<0,050	<0,050	<0,050	<0,050	<0,050	0,050
Soybean	S1	S2	S3	S4	S5	LQ
Glyphosate+Ampa	<	<	<	<	<	
	200,000000	200,000000	200,000000	200,000000	200,000000	200
2,4D+2,4,5T	<0,50	<0,50	<0,50	<0,50	<0,50	0,5
Alaclor	<0,100	<0,100	<0,100	<0,100	<0,100	0,1
Aldicarb + Aldicarbsulfone+aldicarbsulfoxide	<10	<10	<10	<10	<10	10
Aldrin+Dieldrin	<0,00100	<0,00100	<0,00100	<0,00100	<0,00100	0,001
Atrazine	<0,200000	<0,200000	<0,200000	<0,200000	<0,200000	0,2
Carbendazine+Benomil	<20,000	<20,000	<20,000	<20,000	<20,000	20
Carbofuran	<5,000	<5,000	<5,000	<5,000	<5,000	5
ChlordanCis + Trans	<0,005	<0,005	<0,005	<0,005	<0,005	0,005
Chlorpyrifos+ChlorpyrifosOxon	<0,300000	<0,300000	<0,300000	<0,300000	<0,300000	0,3
Diuron	<50,000	<50,000	<50,000	<50,000	<50,000	50
EndosulfanAlpha+EndosulfanBeta+Endosulfan						
Sulfate	<0,010	<0,010	<0,010	<0,010	<0,010	0,01
Endrin	<0,001000	<0,001000	<0,001000	<0,001000	<0,001000	0,001
Gamma-BHC (Lindano)	<0,005000	<0,005000	<0,005000	<0,005000	<0,005000	0,005
Methamidophos	<0,500000	<0,500000	<0,500000	<0,500000	<0,500000	0,5
Molinate	<0,100	<0,100	<0,100	<0,100	<0,100	0,1
o,p-DDD+ o,p-DDE+ o,p-DDT	<0,001000	<0,001000	<0,001000	<0,001000	<0,001000	0,001
MethylParathion	<0,050000	<0,050000	<0,050000	<0,050000	<0,050000	0,05

Pendimethalin	< 0,020000	< 0,020000	< 0,020000	< 0,020000	< 0,020000	0.02
Permethrin	< 0,200	< 0,200	< 0,200	< 0,200	< 0,200	0.2
Profenophos	< 0,050000	< 0,050000	< 0,050000	< 0,050000	< 0,050000	0.05
Simazine	< 0,050000	< 0,050000	< 0,050000	< 0,050000	< 0,050000	0.05
Tebuconazole	< 0,010000	< 0,010000	< 0,010000	< 0,010000	< 0,010000	0.01
Terbuphos	< 0,020000	< 0,020000	< 0,020000	< 0,020000	< 0,020000	0.02
Trifluralin	< 0,020000	< 0,020000	< 0,020000	< 0,020000	< 0,020000	0.02
Sugarcane	S1	S2	S3	S4	S5	LQ
Temperature	28.5	34.1	37	32.2	30.8	
pH	7.7	6.2	8.2	7.3	6.9	
Conductivity	37.1	14.8	85	597	95.1	
Salinity	0.03	0.02	0.05	0.29	0.06	
Total dissolvedsolids	18.3	7.03	42.1	299	51.5	
Dissolvedoxygen	5.1	11.6	14.1	20.1	14.8	
Resistivity	27.4	71	11.8	1.6	9.6	
Total Nitrogen	0.045	< 0,01	0.012	0.056	0.022	0.01
Total Phosphorus	0.02	0.01	0.02	0.18*	0.15*	0.01
2,4D+2,4,5T	<1,15	<1,15	<1,15	<1,15	<1,15	1.15
Alaclor	<0,100	<0,100	<0,100	<0,100	<0,100	0.1
Aldicarb + Aldicarbsulfone + aldicarbsulfoxide	<10	<10	<10	<10	<10	10
Aldrin+Dieldrin	<0,00200	<0,00200	<0,00200	<0,00200	<0,00200	0.002
Atrazine	<1,00	<1,00	<1,00	<1,00	<1,00	1
Carbendazine+Benomil	<20,000	<20,000	<20,000	<20,000	<20,000	20
Carbofuran	<5,000	<5,000	<5,000	<5,000	<5,000	5
ChlordanCis + Trans	<0,020	<0,020	<0,020	<0,020	<0,020	0.02
Chlorpyrifos+ChlorpyrifosOxon	<5,100	<5,100	<5,100	<5,100	<5,100	5.1
Diuron	<50,000	<50,000	<50,000	<50,000	<50,000	50
Endosulfan	<0,030	<0,030	<0,030	<0,030	<0,030	0.03
Alpha+EndosulfanBeta+Endosulfan Sulfate	<0,030	<0,030	<0,030	<0,030	<0,030	
Endrin	<0,001000	<0,001000	<0,0001000	<0,001000	<0,001000	0.001
Gamma-BHC (Lindano)	<0,010	<0,010	<0,010	<0,010	<0,010	0.01
Methamidophos	<5,0000	<5,0000	<5,0000	<5,0000	<5,0000	5
Molinate	<0,100	<0,100	<0,100	<0,100	<0,100	0.1
p,p'DDD+ p,p-DDE+ p,p-DDT	<0,0030	<0,0030	<0,0030	<0,0030	<0,0030	0.003
MethylParathion	<0,050	<0,050	<0,050	<0,050	<0,050	0.05
Pendimethalin	<0,100	<0,100	<0,100	<0,100	<0,100	0.1
Permethrin	<0,200	<0,200	<0,200	<0,200	<0,200	0.2
Profenophos	<0,100	<0,100	<0,100	<0,100	<0,100	0.1
Simazine	<0,100	<0,100	<0,100	<0,100	<0,100	0.1
Tebuconazole	<0,100	<0,100	<0,100	<0,100	<0,100	0.1
Terbuphos	<0,100	<0,100	<0,100	<0,100	<0,100	0.1
Trifluralin	<0,050	<0,050	<0,050	<0,050	<0,050	0.05
Chlomazone	<0,1	<0,1	<0,1	<0,1	<0,1	0.1
Mancozeb	<106,800	<106,800	<106,800	<106,800	<106,800	106.8
Metallochlor	<0,100	<0,100	<0,100	<0,100	<0,100	0.1
Glyphosate + AMPA	<100	<100	<100	<100	<100	100

* Indicates increase above Brazilian legislation; LQ = Limit of quantification; NR: There is no recommendation by law; S = sampling site; The number in front of the "S" reports the sampling site. CONAMA Resolution 357/2005 available at: <http://conama.mma.gov.br/>. Accessed on February 13, 2021

Anexo I

Mamíferos voadores em regiões com degradação crônica do Cerrado

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Resumo A degradação do Cerrado causada especialmente pela expansão das atividades agrícolas, tem sido a principal ameaça a biodiversidade. No presente estudo avaliamos a composição de morcegos em regiões agrícolas e em uma Unidade de Conservação, ambos no Cerrado Goiano. Além disso, utilizamos o teste de micronúcleo como biomarcador para comparar dano no DNA entre áreas agrícolas e conservadas para a espécie *Phyllostomus discolor*. Um total de 487 morcegos de 18 espécies pertencentes a três famílias (Phyllostomidae, Molossidae, e Vespertilionidae) foram registrados. As espécies mais comuns foram *Glossophaga soricina* (n = 91), *Carollia perspicillata* (n = 86), *Artibeus planirostris* (n = 79), *Artibeus lituratus* (n = 61) e *Platyrrhinus lineatus* (n = 46). A média de vegetação nativa extraída em áreas agrícolas foi de 79,21% a menos comparado a Unidade de Conservação. Através da análise de amostras de células esfoliadas da mucosa bucal de *P. discolor* observamos dano no DNA (micronúcleo e broto nuclear), distúrbio mitótico (binucleadas) e citotoxicidade (célula picnótica, cariorrexi e cariólise). Finalmente, esse estudo fortalece a compreensão das espécies de morcegos que vivem em áreas dominadas pela agricultura no cerrado goiano e sua susceptibilidade de dano genético.

Palavras-chave: Quirópteros, savana brasileira, degradação crônica, bioindicadores

Introdução

O desmatamento tem proporcionado a perda e fragmentação de habitats naturais fortalecendo para uma crise global da biodiversidade (Ramalho et al., 2022). Hotspots de biodiversidade como o Cerrado, têm experimentado mudanças de uso e cobertura da terra, apresentando altas taxas de desmatamento devido a expansão agrícola, com aproximadamente 50% de sua vegetação natural já removida (Alurralde e Díaz 2021; Matosak et al., 2022). A degradação dos habitatsleva à redução no número de espécies e, consequentemente, a mudanças na estrutura e estabilidade das redes de interações (Laurindo et al., 2019) provocando desequilíbrio nas comunidades de muitos grupos de animais, como por exemplo dos morcegos.

Na região Neotropical os morcegos representam quase 50% de todas as espécies de mamíferos, atingindo uma grande diversidade taxonômica e funcional (Alurralde e Díaz 2021). No Brasil existem 181 espécies (Garbino et al., 2020), sendo 118 registradas no Cerrado (Aguiar et al., 2016), e 90 no estado de Goiás (Hannibal et al., 2021). Embora o estado abrigue quase a metade da diversidade de espécies (49,72%) de morcegos do Brasil, muitas regiões ainda são pouco amostradas, sobretudo aquelas com intensas atividades econômicas agroindustriais. Dentre as múltiplas ameaças aos morcegos estão a perda e fragmentação de habitats naturais (Weier et al., 2021), poluentes químicos (de Souza et al., 2020) e doenças (Gómez-Rodríguez et al., 2022). Portanto, estudos que visam à compreensão das espécies locais são importantes para elaboração de medidas de conservação.

Os morcegos podem ser considerados excelentes bioindicadores para estimar a resposta da qualidade ambiental (Bobrowiec et al., 2022; Sotero et al., 2022), seja em nível celular (xenobióticos) ou de comunidade. Eles também são considerados eficazes na restauração de áreas degradadas, pois são importantes dispersores de sementes e polinizadores de flores (Muylaert et al., 2016). Desse modo, o presente estudo teve como objetivo conhecer a riqueza de morcegos em remanescentes do cerrado em regiões do Sudoeste Goiano (município de Rio Verde e Parque Nacional das Emas) e Centro Oeste do estado em Paraúna, em Goiás. Cada ambiente também foi quantificado a porcentagem de vegetação nativa em relação ao uso e ocupação do solo. Adicionalmente, aplicamos o teste de micronúcleo em amostras de células esfoliadas da mucosa bucal de *Phyllostomus discolor* para avaliar o estado de saúde dos morcegos nos diferentes sítios amostrais.

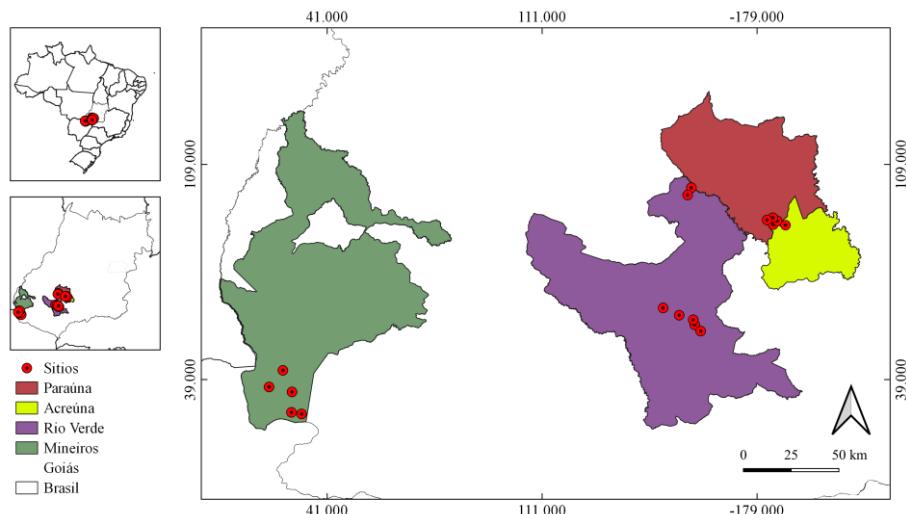
Material e Métodos

Área de estudo e amostragem

Os morcegos foram amostrados entre Junho de 2016 a Novembro de 2021 no Cerrado Goiano. Em Paraúna/Acreúna (fragmentos florestais em área de cana de açúcar) e Parque Nacional das Emas as capturas foram realizadas entre Julho de 2019 a Novembro de 2021 (Figura 1). Os animais foram amostrados por meio de redes de neblina. Cinco pontos de coletas foram amostrados em área de cana e Parque Nacional das Emas (PNE) onde as redes tinham 12 m x 2,5 m, colocadas 50 cm acima do solo e ficando abertas do pôr do sol até as 22 em um total de 10 dias campo em cada área ($2.400 \text{ m}^2 \cdot \text{h}$ rede). Já em Rio Verde as coletas ocorrem entre as 18h até as 00h em sete áreas somando 84 dias ($15.120 \text{ m}^2 \cdot \text{h}$).

Em cada sítio de amostragem, atributos da paisagem como a porcentagem de vegetação nativa foi extraída em um raio de 1 km do centróide da coleta. Malhas do Mapbiomas para o ano de estudo foi utilizado para a análise da vegetação conduzido no QGis. Baseado em nossos estudos anteriores (Benvindo-Souza et al., 2021,2022), morcegos fêmeas gestantes ou com filhotes eram coletados nas redes e soltas e os demais animais foram colocados em sacos de algodão e identificados baseado em Reis et al. (2013). Segundo os requisitos legais de pesquisa, o estudo foi aprovado pelo Comitê de Ética no Uso de Animais do Instituto Federal de Goiano (CEUA: 8436060516) e da Universidade Federal de Goiás (n.30/21), bem como pelo Instituto Chico Mendes de Conservação da Biodiversidade (n. 54101-1 e n. 69513-2).

Fig. 1 Municípios amostrados para a fauna de morcegos em Goiás, Brasil.



Em Rio Verde, uma amostra *Phyllostomus discolor* foi obtidas amostras de células esfoliadas pela primeira vez no sentido de expandir o uso do teste de micronúcleo em morcegos. Após a obtenção das amostras os animais foram soltos no mesmo local de captura. Utilizando um cotonete (swab), esfregamosa mucosa lateral da bochecha, o fundo e as

gengivas da boca dos morcegos, conforme Benvindo-Souza et al. (2019; 2022). As células da mucosa oral foram transferidas para quatro lâminas de vidro a qual continha uma gota de solução salina. Após secas a temperatura ambiente foi fixada em solução de metanol 100% por 10 minutos. Concomitantes, após secas foram coradas com Panoptic Rápido mergulhando as lâminas cinco vezes em cada solução e depois enxaguando com água destilada para remover o excesso de solução de corante. Um total de mil células por indivíduo foram contadas sob um microscópio óptico com ampliação de $\times 100$. Quantificamos Micronúcleo e outras anormalidades como broto nuclear, binucleação, cariorrexe, picnose e cariólise.

Análise de dados

A dominância foi determinada pelo índice de Dominância Berger-Parker. Foi comparada a porcentagem de vegetação nativa por meio de Kruskal-Wallis (H). O teste U de Mann-Whitney foi usado para a comparação entre morcegos no PNE e cana-de-açúcar. Uma correlação de Pearson ou Spearman dependendo da distribuição dos dados foi realizada entre micronúcleo e outras anormalidades nucleares. O status de conservação das espécies foi obtidona lista brasileira de fauna ameaçada de extinção MMA (2022) e a lista internacional da IUCN (2022).

Resultados

Riqueza de espécies de morcegos

Um total de 487 morcegos de 18 espécies foram amostrados no presente estudo. Os animais pertenciam a três famílias cujo Phyllostomidae detiveram maior frequência de capturas (88%), seguido por Molossidae (8%) e Vespertilionidae (4%). As espécies foram obtidas em três regiões (Tabela 1 e 2), no Parque Nacional das Emas ($n = 11$ espécies), Área de cana-de-açúcar ($n = 9$) e soja que deteve maior riqueza ($n = 16$).

No geral os maiores esforços de captura (Figura 3) foram registrados para *Glossophaga soricina*, mais abundante ($n = 91$), *Carollia perspicillata* ($n = 86$), *Artibeus planirostris* ($n = 79$), *Artibeus lituratus* ($n = 61$) e *Platyrrhinus lineatus* ($n = 46$). Em Rio Verde a dominância foi de *Artibeus planirostris* ($n = 73$), *Artibeus lituratus* ($n = 56$), *Carollia perspicillata* ($n = 44$) e *Platyrrhinus lineatus* ($n = 43$). Foi observado que as espécies pertenciam a cinco guildas tróficas tendo os frugívoros com maior esforço de captura ($n = 283$; 58.11%), seguido por ($n = 93$; 19.10%), Onívoro ($n = 50$; 10.27%), carnívoro ($n = 1$; 0.21%) e insetívoro ($n = 60$; 12.32%).

Tabela 1 Espécies de morcegos registrados em remanescentes florestais do cerrado em Goiás, Brasil.

Espécies	PNE	Cana	Soja	Abundância	Guilda	Status
Phyllostomidae						
Carollinae						
<i>Carollia perspicillata</i> (Linnaeus, 1758)	6	36	44	86(17.66%)	F	LC
Glossophaginae						
<i>Glossophaga soricina</i> (Pallas, 1766)	52	3	36	91(18.69%)	N	LC
<i>Anoura caudifer</i> (É. Geoffroy, 1818)	1	1		2(0.41%)	N	LC
Stenodermatinæ						
<i>Artibeus lituratus</i> (Olfers, 1818)	4	1	56	61(12.53%)	F	LC
<i>Artibeus planirostris</i> (Spix, 1823)	6		73	79(16.22%)	F	LC
<i>Artibeus cinereus</i> (Gervais, 1856)			2	2(0.41%)	F	LC
<i>Platyrrhinus incarum</i> (Thomas, 1912)			2	2(0.41%)	F	LC
<i>Platyrrhinus lineatus</i> (É. Geoffroy, 1810)	2	2	42	46(9.45%)	F	LC
<i>Sturnira lilium</i> (É. Geoffroy, 1810)	4		3	7(1.44%)	F	LC
Phyllostominae						
<i>Phyllostomus discolor</i> Wagner, 1843			10	10(2.05%)	O	LC
<i>Phyllostomus hastatus</i> (Pallas, 1767)		17	23	40(8.21%)	O	LC
<i>Chrotopterus auritus</i> (Peters, 1856)	1			1(0.21%)	C	LC
Molossidae						
<i>Eumops maurus</i> (Thomas, 1901)			2	2(0.41%)	I	DD
<i>Molossops temmincki</i> (Burmeister, 1854)		1	9	10(2.05%)	I	LC
<i>Molossus molossus</i> Pallas, 1766	16		11	27(5.54%)	I	LC
Vespertilionidae						
<i>Lasiurus blossevillii</i> ([Lesson, 1826])		1	1	2(0.41%)	I	LC
<i>Myotis albescens</i> (É. Geoffroy, 1806)	4	2	8	14(2.87%)	I	LC
<i>Myotis nigricans</i> (Schinz, 1821)	1		4	5(1.03%)	I	LC
Abundância total	97	64	326	487		
Riqueza		11	9	16		

PNE: Parque Nacional das Emas. Guilda trófica: I) Insetívora, F) Frugívoro, N) Nectárvoro e O) Onívoro. Status conforme a IUCN: LC) Pouco preocupante, DD) Deficiente de dados.

Das espécies registradas, *Eumops maurus* é classificada na IUCN como deficiente de dados, e as demais espécies são pouco preocupantes nessa lista internacional. Nenhuma das espécies se encontram ameaçadas conforme a lista brasileira (MMA, 2022). A porcentagem da vegetação nativa (Figura 2) nas áreas agrícolas foram inferiores a 21% em relação à Unidade de Conservação ($H = 10,0156$; $p = 0,006$). No PNE e na área de cana-de-açúcar onde houve

padronização nas amostragens em dias de campo não houve diferença significativa na riqueza/abundância de indivíduos ($p = 0,342$).

Fig. 2 Porcentagem média de cobertura vegetal nas áreas de estudo.

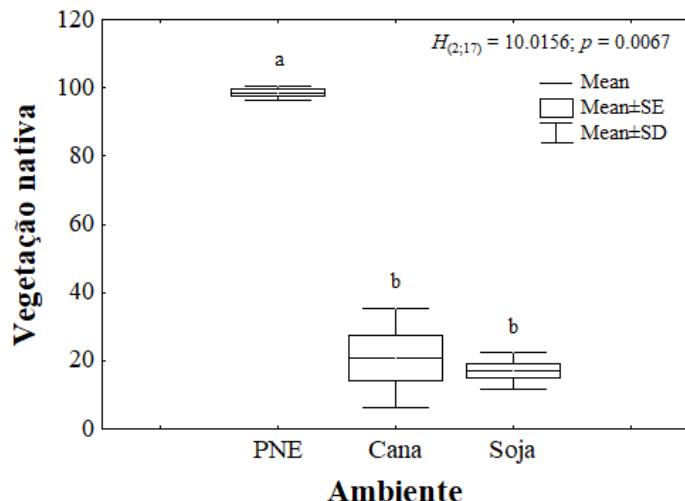


Fig. 3 Algumas espécies de morcegos amostrados em áreas alteradas e conservadas do Cerrado no estado de Goiás, Brasil.



Morcegos como bioindicadores de qualidade ambiental

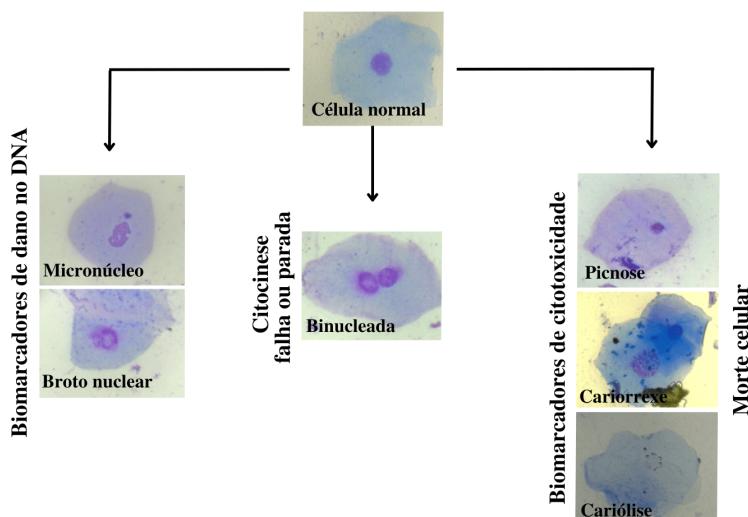
Amostras de células esfoliadas foram obtidas para representar danos em células como lesão no DNA, distúrbio mitótico e citotoxicidade em *Phyllostomus discolor* como caráter representativo (Tabela 2). A frequência de micronúcleo foi de 0.14 micronúcleo para mil células analisadas. Não houve correlação entre micronúcleo e demais anormalidade nucleares.

Tabela 3 Micronúcleo e outras anormalidades nucleares em *Phyllostomus discolor*.

MN e ANs	Media±Erro padrão	Correlação	p-valor
Micronúcleo	0.14±0.04		
Broto nuclear	0.21±0.06	-0.23	0.518
Binucleada	0.25±0.04	0.04	0.894
Picnose	1.10±0.40	-0.47	0.17
Cariorrexia	2.21±0.38	0.34	0.33
Cariólise	0.03±0.02	0.15	0.66

Fig. 4 Fotomicrografias de células esfoliadas de mucosa bucal de um morcego *Phyllostomus discolor* indicando as imagens em cor padrão e seus respectivos negativos.

Teste de micronúcleo em células esfoliadas de mucosa bucal



Discussão

Neste estudo apresentamos uma riqueza de espécies de morcegos em fragmentos florestais em Rio Verde Goiás, Paraína/Acreúna e Parque Nacional das Emas. Foram amostradas 18 espécies, o que corresponde a 15,25% das espécies encontradas no Cerrado. Em Rio Verde, uma região de cultivo de soja, foi observada que a cobertura de vegetação nativa era de 17% e na área de cana-de-açúcar de 20%. Dessa forma, indicaram significativamente menor em cobertura vegetal em relação à área de referência, PNE. A família Phyllostomidae foi a mais diversa corroborando com a lista de morcegos de Goiás (Hannibal et al., 2021), sendo uma resposta esperada em razão ao método de captura (rede de neblina), e por ser a família mais diversa do país. Estudos com morcegos nas regiões do presente estudo ainda são escassos especialmente trabalhos que buscam descrever a composição de espécies. Tendo em vista que Morais et al. (2022) estudaram multítaxons em Rio Verde, esse estudo com quirópteros complementa o reconhecimento da fauna local.

O PNE é a maior área protegida do Cerrado, declarada como Patrimônio Mundial Natural pela Unesco (IPHAN 2022). Nessa unidade de conservação foram registrado 19 espécies por Rodrigues et al. (2002) e 11 espécies no presente estudo, sendo três novos registros para o local, *Anoura caudifer*, *Myotis albescens* e *Myotis nigricans* elevando a diversidade da área para 22 espécies. *Glossophaga soricina* foi a mais abundante no PNE, isso ocorreu em razão às redes de neblina serem instaladas em vegetação próxima a construções humanas que por vez serviam de abrigo para colônias. Embora o Parque abrigue uma considerável fauna de morcegos, os principais riscos locais são as extensas lavouras do seu entorno. Modificações nos habitats ao redor de áreas protegidas também já foi relatado por Oliveira et al. (2017), e pode ter implicações na fauna isolada nessas ilhas. O presente estudo adiciona novas espécies aos municípios de Rio Verde e Paraúna que também foram pouco estudados (Benvindo-Souza et al., 2019, 2022), também observado na lista de mamíferos de Goiás (Hannibal et al., 2021).

Para Rio Verde, a área de soja, a maior abundância e riqueza de espécie ocorreram devido ao elevado esforço amostral quando comparado as demais regiões. No entanto, mais espécies insetívoras poderiam ter sido amostradas nesse município se o método acústico tivesse sido complementado. Estrada Villegas et al. (2015) ressalva que para os morcegos neotropicais, os insetívoros aéreos têm sido sistematicamente subamostrados porque evitam redes de neblina, a ferramenta de amostragem tradicional. As espécies dominantes foram principalmente *Glossophaga soricina*, *Artibeus lituratus* e *A. planirostris*. Zortéia et al. (2008) também encontrou grande abundância de *G. soricina* no Sudoeste Goiano. Sendo estes animais frequentemente capturados em toda a região neotropical (Calderón et al., 2019; Benvindo-Souza et al., 2021). Espécies como *Phyllostomus discolor*, *Artibeus cinereus*, *Platyrrhinus incarum* e *Eumops maurus* são conhecidas somente no município de Rio Verde, em Goiás, apresentando baixa abundância. Cada espécie de morcegos responde de forma diferente à alteração dos habitats (Alurralde e Díaz 2021), onde alguns podem ser mais generalistas, enquanto outros são mais sensíveis a perturbação ambiental.

No que se refere ao hábito alimentar, fragmentos remanescentes do cerrado especialmente aqueles de área agrícola abrigava principalmente espécies frugívoras, insetívoras, onívoras e nectarívoras que reflete em serviços ecossistêmicos. Serviços ambientais são extremamente importantes para os processos de sucessão florestal, regeneração e maturação de florestas secundárias em toda a região Neotropical (Brändel et al., 2020), bem como controle de populações de insetos. A área de estudo apresentou baixa riqueza de espécies carnívora, com apenas o registro de *Chrotopterus auritus*, concordando com o estudo de

Zortéia et al.(2021). Nas áreas selecionadas para o estudo não registramos espécies hematófagas, mas a espécie foi documentada anteriormente no PNE (Rodrigues et al., 2002).

No presente estudo não houve aumento de outras anormalidades nucleares em relação a micronúcleo. O uso recente dessa técnica em morcegos está sendo aplicada principalmente em amostras de sangue e células esfoliadas de mucosa bucal (Sotero et al., 2022). Através das células esfoliadas é possível diagnosticar a presença dano no DNA (micronúcleo e broto nuclear), distúrbio mitótico (célula binucleada), e dano citotóxico como a presença de células cariorraxe, picnose e cariólise, que é indicativo de morte celular, assim como demonstrado no presente estudo. Núcleo com cromatina condensada também podem ser pontuadas como resposta de citotoxicidade (Benvindo-Souza et al., 2019), no entanto com cautela na sua confirmação, de modo a não confundir a alteração nuclear com resíduos de corante.

Pesquisas anteriores mostraram que populações de morcegos em áreas com paisagens afetadas pela agricultura detinham maior frequência de dano no DNA em relação a áreas conservadas (Benvindo-Souza et al., 2019, 2022; Sandoval-Herrera et al., 2021). Uma série de estudos de diferentes táxons incluindo os humanos tem evidenciado a validação do teste de micronúcleo para a sensibilidade em expressão de dano mutagênico diante da exposição a agrotóxicos (Kornilova et al., 2022; Verma et al., 2022; Rutkoski et al., 2022), inclusive em humanos (Malarcane et al., 2022). Considerando as vantagens do teste de micronúcleo, ele pode ser uma ferramenta adicional no fornecimento de um quadro mais abrangente do estado de saúde dos animais que vivem em comunidades (Santovito et al., 2022) em especial aos morcegos, devido sua plasticidade em habitar diferentes ambientes.

Em síntese, neste estudo 18 espécies foram amostradas no estado de Goiás. Considerando somente fragmentos remanescentes no cerrado sobre a influência da agricultura, 16 espécies foram registradas em um total 94 dias de captura. Dessa forma, foi considerada baixa a riqueza de espécies e que mais dias nas áreas de cana-de-açúcar e PNE poderia ter alavancando a riqueza de animais. Para o PNE mais estudos voltados a morcegos são necessários, haja vista que para nosso conhecimento esse é o terceiro trabalho na área.

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Conflito de interesse

Os autores declaram não haver conflito de interesse

Referências

- Aguiar, L. M., Bernard, E., Ribeiro, V., Machado, R. B., & Jones, G. (2016). Should I stay or should I go? Climate change effects on the future of Neotropical savannah bats. *Global Ecology and Conservation*, 5, 22-33.
- Alurralde, S. G., & Díaz, M. M. (2021). Assemblage-level responses of Neotropical bats to forest loss and fragmentation. *Basic and Applied Ecology*, 50, 57-66. <https://doi.org/10.1016/j.baae.2020.09.001>
- Benvindo-Souza M, Hosokawa AV, Dos Santos CGA, de Assis RA, Pedroso TA, Borges RE, Pacheco SM, Souza Santos LR, Silva DDM (2022) Evaluation of genotoxicity in bat species found on agricultural landscapes of the Cerrado savanna, central Brazil. *Environ Pollut* 118579. <https://doi.org/10.1016/j.envpol.2021.118579>
- Benvindo-Souza, M., de Souza Santos, L. R., Elias Borges, R., Alves de Assis, R., de Melo e Silva, D., Zortea, M., & Missel Pacheco, S. (2021). Thousands of bats: A portrait of the chiropteran fauna of Palmas city, Central Brazil. *Austral Ecology*, 46(5), 876-879. <https://doi.org/10.1111/aec.13032>
- Benvindo-Souza, M., Borges, R. E., Pacheco, S. M., & de Souza Santos, L. R. (2019). Genotoxicological analyses of insectivorous bats (Mammalia: Chiroptera) in central Brazil: The oral epithelium as an indicator of environmental quality. *Environmental Pollution*, 245, 504-509. <https://doi.org/10.1016/j.envpol.2018.11.015>
- Brändel, S. D., Hiller, T., Halczok, T. K., Kerth, G., Page, R. A., & Tschapka, M. (2020). Consequences of fragmentation for Neotropical bats: The importance of the matrix. *Biologicalconservation*, 252, 108792. <https://doi.org/10.1016/j.biocon.2020.108792>
- Bobrowiec, P. E. D., Farneda, F. Z., Nobre, C. C., & da Cunha Tavares, V. (2022). Taxonomic and functional responses of bats to habitat flooding by an Amazonian mega-dam. *Biodiversity and Conservation*, 1-19. <https://doi.org/10.1007/s10531-022-02396-8>
- Calderón, A., Guzmán, C., Mattar, S., Rodriguez, V., Martínez, C., Violet, L., Martínez, J. & Figueiredo, L. T. M. (2019). Dengue virus in bats from Córdoba and Sucre, Colombia. *Vector-Borne and Zoonotic Diseases*, 19(10), 747-751. <https://doi.org/10.1089/vbz.2018.2324>
- de Oliveira, H. F., de Camargo, N. F., Gager, Y., & Aguiar, L. M. (2017). The response of bats

- (Mammalia: Chiroptera) to habitat modification in a Neotropical Savannah. *Tropical Conservation Science*, 10, 1940082917697263. <https://doi.org/10.1177/1940082917697263>
- de Souza, M. B., de Souza Santos, L. R., Borges, R. E., Nunes, H. F., Vieira, T. B., Pacheco, S. M., & de Melo e Silva, D. (2020). Current status of ecotoxicological studies of bats in Brazil. *Bulletin of environmental contamination and toxicology*, 104(4), 393-399. <https://doi.org/10.1007/s00128-020-02794-0>
- Estrada Villegas, S., Meyer, C. F., McGill, B., & Kalko, E. K. (2015). Assessing the structure of a Neotropical bat community using acoustic monitoring techniques. *The Journal of the Acoustical Society of America*, 138(3), 1905-1905. <https://doi.org/10.1121/1.4933991>
- Garbino, G.S.T., R. Gregorin, I.P. Lima, L. Loureiro, L.M. Moras, R. Moratelli, M.R. Nogueira, A.C. Pavan, V.C. Tavares, M.C. do Nascimento and A.L. Peracchi. 2020. Updated checklist of Brazilian bats: versão 2020. Comitê da Lista de Morcegos do Brasil—CLMB. Sociedade Brasileira para o Estudo de Quirópteros (Sbeq).<<https://www.sbeq.net/lista-de-especies>> acessado em: 26 de Outubro de 2022.
- Gómez-Rodríguez, R. A., Sánchez-Cordero, V., Boyer, D., Schondube, J. E., Rodríguez-Moreno, Á., & Gutiérrez-Granados, G. (2022). Risk of infection of white-nose syndrome in North American vespertilionid bats in Mexico. *Ecological Informatics*, 101869. <https://doi.org/10.1016/j.ecoinf.2022.101869>
- Kornilova, A. A., Zhapbasov, R. Z., Zhomartov, A. M., Sibataev, A. K., Begimbetova, D. A., & Bekmanov, B. O. (2022). Genotoxic Effect of Unused and Banned Pesticides on the Body of Cattle Kept on the Territory of South Kazakhstan. *Contemporary Problems of Ecology*, 15(2), 180-187. <https://doi.org/10.1134/S1995425522020044>
- Laurindo, R. S., Novaes, R. L. M., Vizentin-Bugoni, J., & Gregorin, R. (2019). The effects of habitat loss on bat-fruit networks. *Biodiversity and Conservation*, 28(3), 589-601. <https://doi.org/10.1007/s10531-018-1676-x>
- Malacarne, I. T., Takeshita, W. M., de Souza, D. V., dos Anjos Rosario, B., de Barros Viana, M., Renno, A. C. M., Salvadori, D. M. V., Ribeiro, D. A. (2022). Is micronucleus assay in oral exfoliated cells a useful biomarker for biomonitoring populations exposed to pesticides? A systematic review with meta-analysis. *Environmental Science and Pollution Research*, 1-12. <https://doi.org/10.1007/s11356-022-22015-x>
- Matosak, B. M., Fonseca, L. M. G., Taquary, E. C., Maretto, R. V., Bendini, H. D. N., & Adami, M. (2022). Mapping Deforestation in Cerrado Based on Hybrid Deep Learning Architecture and Medium Spatial Resolution Satellite Time Series. *Remote sensing*, 14(1), 209. <https://doi.org/10.3390/rs14010209>

- Morais, A. R., Freitas-Oliveira, R., Moreira, J. C., Souza, A. O. D., Bittar, B. B., Carvalho, F. M. V. D., Oliveira GV, Santos LRS, Guimarães MA, Amorim NPL, Assis RA, Borges RE, Oliveira SR, Adreani TL, Siqueira MN, Siqueira, M. N. (2022). Multi-taxon inventory and landscape characterization in an agrosystem of the Brazilian Midwest targeted for payment for environmental services. *Biota Neotropica*, 22. <https://doi.org/10.1590/1676-0611-BN-2021-1283>
- Muylaert, R. L., Stevens, R. D., & Ribeiro, M. C. (2016). Threshold effect of habitat loss on bat richness in cerrado-forest landscapes. *Ecological Applications*, 26(6), 1854-1867. <https://doi.org/10.1890/15-1757.1>
- MMA (2022) Ministério do Meio Ambiente: Lista oficial das espécies da fauna brasileira ameaçadas de extinção, Disponível em: <https://www.sbeq.net/>. Acesso em 25 de Outubro de 2022
- IPHAN, 2022. Reservas do Cerrado: Parques Nacionais da Chapada dos Veadeiros e das Emas (GO). Disponível em: <http://portal.iphan.gov.br/pagina/detalhes/53>. Acesso em: 24 de Outubro de 2022
- Garbino G.S.T., Gregorin R., Lima I.P., Loureiro L., Moras L., Moratelli R., Nogueira M.R., Pavan A.C., Tavares V.C., Nascimento M.C., Novaes, R.L.M., Peracchi A.L. 2023. Updated checklist of Brazilian bats: versão 2020. Comitê da Lista de Morcegos do Brasil—CLMB. Sociedade Brasileira para o Estudo de Quirópteros (Sbeq).<<https://www.sbeq.net/lista-de-especies>>. Acessado em: 24 de Fevereiro de 2023
- Hannibal, W., Zortéa, M., Calaça, A.M., Carmignotto, A.P., Bezerra, A.M.R., Carvalho, H.G., Bonvicino, C.R., Martins, A.C.M., Aguiar, L.M.S., de Souza, M.B., Mattos, I., Oliveira, R.F., Brito, D., Silva, D.A., Guimarães, M.A., do Carmo, E.M.B., Moreira, J.C. Checklist of mammals from Goiás, central Brazil. *Biota Neotropica* 21(3): e20201173. <https://doi.org/10.1590/1676-0611-BN-2020-1173>
- Ramalho, W. P., With, K. A., de Sousa Mesquita, G., de Arruda, F. V., Guerra, V., Ferraz, D., Andrade, M. S., do Prado, V. H. M. (2022). Habitat fragmentation rather than habitat amount or habitat split reduces the diversity and abundance of ground-dwelling anurans within forest remnants of the Brazilian Cerrado. *Journal for Nature Conservation*, 126259. <https://doi.org/10.1016/j.jnc.2022.126259>
- Reis NR, Fregonezi MN, Peracchi AL, Shibatta OA (2013) Morcegos do Brasil: Guia de Campo. Rio de Janeiro: Technical Books. Londrina
- Rodrigues FHG, Silveira L, Jácomo AT, Carmignotto AP, Bezerra AMR, Coelho DC, Garbogini H, Pagnozzi J, Hass A (2002) Composição e caracterização da fauna de

mamfferos do Parque Nacional das Emas, Goias, Brasil. Revta bras. Zool. 19 (2): 589 - 600, 2002

Rutkoski, C. F., Grott, S. C., Israel, N. G., Carneiro, F. E., de Campos Guerreiro, F., Santos, S., Horn P.A., Trentini, A.A., Silva, E.B., Albuquerque, C.A., Alves T.C., de Almeida, E. A. (2022). Hepatic and blood alterations in *Lithobatescatesbeianus* tadpoles exposed to sulfamethoxazole and oxytetracycline. Chemosphere, 307, 136215. <https://doi.org/10.1016/j.chemosphere.2022.136215>

Sandoval-Herrera, N., Paz Castillo, J., Herrera Montalvo, L. G., & Welch Jr, K. C. (2021). Micronucleus test reveals genotoxic effects in bats associated with agricultural activity. Environmental Toxicology and Chemistry, 40(1), 202-207. <https://doi.org/10.1002/etc.4907>

Santovito, A., Buglisi, M., & Sciandra, C. (2022). Buccal micronucleus assay as a useful tool to evaluate the stress-associated genomic damage in shelter dogs and cats: new perspectives in animal welfare. Journal of Veterinary Behavior, 47, 22-28. <https://doi.org/10.1016/j.jveb.2021.09.007>

Sotero, D. F., Benvindo-Souza, M., de Freitas, R. P., & e Silva, D. D. M. (2022). Bats and pollution: Genetic approaches in ecotoxicology. Chemosphere, 307, 135934. <https://doi.org/10.1016/j.chemosphere.2022.135934>

Verma, S. K., Soni, R., & Gupta, P. (2022). Chloroacetamide herbicide pretilachlor induces genotoxicity in the fresh water fish *Clariasbatrachus*. Toxicological & Environmental Chemistry, 104(1), 120-128. <https://doi.org/10.1080/02772248.2021.2007921>

Weier, S. M., Linden, V. M., Hammer, A., Grass, I., Tscharntke, T., & Taylor, P. J. (2021). Bat guilds respond differently to habitat loss and fragmentation at different scales in macadamia orchards in South Africa. Agriculture, Ecosystems & Environment, 320, 107588. <https://doi.org/10.1016/j.agee.2021.107588>

Zortéa, M., & Alho, C. J. (2008). Bat diversity of a Cerrado habitat in central Brazil. Biodiversity and Conservation, 17(4), 791-805. <https://doi.org/10.1007/s10531-008-9318-3>

Zortéa, M., de Souza Gomes, K., Tomaz, L. A. G., Palmeirim, J. M., & Lima-Ribeiro, M. S. (2021). Impacts of a hydroelectric power plant on the bat community in central Brazil. Mammal Research, 66(3), 509-518. <https://doi.org/10.1007/s13364-021-00577-4>