UNIVERSIDADE ESTADUAL DE SANTA CRUZ

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E CONSERVAÇÃO DA BIODIVERSIDADE

JULIÁN BARILLARO

BIOACUMULAÇÃO DE METAIS PESADOS EM MORCEGOS DE AGROFLORESTAS DE CACAU SOMBREADO DO NORDESTE DO BRASIL

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia e Conservação da Biodiversidade da Universidade Estadual de Santa Cruz como requisito para obtenção do título de Mestre em Ecologia e Conservação da Biodiversidade

Área de Concentração: Ecologia e conservação de comunidades, ecossistemas e paisagens, Mastozoologia e Ecotoxicologia

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Ilhéus, 23 de junho de 2022.

Dr. Ricardo Siqueira Bovendorp
Universidade Estadual de Santa Cruz (UESC)
(Orientador)

Dr. Fernando Gonçalves
Center for Macroecology, Evolution and Climate
Globe Institute
University of Copenhagen, Denmark (CMEC-UC)
(Membro externo)

Dr. Martín R. Alvarez
Universidade Estadual de Santa Cruz (UESC)
(Membro interno)

B275 Barillaro, Julián.
Bioacumulação de metais pesados em morcegos de ágroflorestas de cacau sombreado do nordeste do Brasil / Julián Barillaro. – Ilhéus, BA: UESC, 2022.
46f. : il.

Orientador: Ricardo Siqueira Bovendorp
Dissertação (Mestrado) – Universidade Estadual de Santa Cruz. Programa de Pós-Graduação em Ecologia e Conservação da Biodiversidade – PPGECB
Inclui referências.

1. Produtos químicos agrícolas. 2. Indicadores biológicos. 3. Chiroptera. 4. Mata Atlântica. 5. Cacau. I. Título.

CDD 632.95

AGRADECIMENTOS

Gostaria de agradecer especialmente a minha mãe, Hemilse e ao meu pai, Raúl, por todo o esforço investido em mim; também ao meu irmão Sebastián e minhas irmãs Valeria e Melisa por tudo que me ensinam no dia a dia. Palavras e linguagens não são suficientes para descrever o amor infinito com que enchem minha vida.

Ao Programa de Pós-Graduação em Ecologia e Conservação da Biodiversidade e seus professores pela base teórica científica e proporcionar momentos intensos de formação acadêmica, CNPq por possibilitar a minha permanência na pesquisa a partir da concessão da bolsa. Muito obrigado a os membros da banca, Ricardo Bovendorp, Fernando Gonçalvez e Martin R. Alvarez, por compartilhar seus conhecimentos comigo e formar parte de meu crescimento cientifico. Especial menção para meu orientador, por me guiar pacientemente ao longo de todo este trabalho, tirando dúvidas e ajudando sempre, sem importar dia nem horário.

Agradeço imensamente a Rebeca Sampaio, Paloma Resende, Maycon Santos, Letícia Costa, Franger García, João Emanoel, Paola De La Quintana, e Wilson Corea pela ajuda, principalmente no trabalho de campo, e a Nicolas Garimano, pela sua orientação na hora de aplicar a bolsa da OEA, além dos valiosos conselhos durante todo o projeto. Também aos proprietários das fazendas por nos permitir trabalhar em suas propriedades, fornecendo logística e informações.

Estou muito grato com os membros do Laboratório de Ecologia Aplicada e Conservação por suas contribuições científicas e de campo. Agradecemos também aos membros do Laboratório de Análise de Tecidos Vegetais da UFPB – Areia/Pb, pela ajuda na análise do pelo. Asimismo, agradezco también a Lio Messi por sus goles, gambetas y por la Copa. Ojalá pueda traernos la otra.

Por fim, quero agradecer a todas as pessoas com quem troquei aprendizado e ensino durante minha vida. O conhecimento é um bem que aumenta quando é compartilhado.

SUMÁRIO

RESUMO

As florestas tropicais abrigam a maior parte da biodiversidade do mundo e cumprem funções-chave para nossa própria sobrevivência. Porém, cada vez mais estão sendo convertidas em terras agrícolas manejadas intensivamente com produtos químicos que podem liberar poluentes perigosos no meio ambiente. Muitos deles contêm metais pesados, elementos extremamente nocivos mesmo em baixas concentrações, pois podem bioacumular nos organismos, com efeitos deletérios nos ecossistemas. Os morcegos, organismos com funções chaves para a manutenção das florestas, são susceptíveis a esse tipo de contaminantes. Pouco se sabe sobre a bioacumulação de metais pesados em morcegos do Brasil e nunca foi avaliada nas agroflorestas sombreadas de cacau do sul da Bahia, ambientes importantes para a conservação que formam parte de uma paisagem agrícola normalmente manejada com agroquímicos. Vários estudos mostraram que pode ser estimada analisando as concentrações em amostras de pelo de morcegos, pois estas se correlacionam com aquelas em órgãos internos e no ambiente. Dessa forma, por meio de uma técnica minimamente invasiva, seria possível conhecer o estado toxicológico dos morcegos, além e acessar a os níveis de contaminação por metais pesados a que podem estar expostos outros seres vivos em perigo. Diversas atividades humanas continuam depositando metais pesados no meio ambiente, portanto, o uso de técnicas de monitoramento eficazes é essencial para planejar e executar soluções baseadas em evidências para os problemas relacionados a esses poluentes.

Palavras-chave: Agrotóxicos, Bioindicador, Chiroptera, Mata Atlântica, *Theobroma cacao*.

ABSTRACT

Tropical forests are home to most of the world's biodiversity and fulfill key functions for our very survival. However, they are increasingly being converted into farmland that is intensively managed with chemicals that can release dangerous pollutants into the environment. Many of them contain heavy metals, extremely harmful elements even in low concentrations, as they can bioaccumulate in organisms, increasing their deleterious effects on ecosystems. Bats, organisms with key functions for the maintenance of forests, are susceptible to this type of contaminants. Little is known about the bioaccumulation of heavy metals in bats from Brazil and has never been evaluated in the shaded cocoa agroforests of southern Bahia, important conservation environments that form part of an agricultural landscape normally managed with agrochemicals. Several studies have shown that it can be estimated by analyzing concentrations in samples of bat fur, as these correlate with those in internal organs and the environment. In this way, through a minimally invasive technique, it would be possible to know the toxicological status of bats, in addition to accessing the levels of contamination by heavy metals to which other living beings in danger may be exposed. Several human activities continue to deposit heavy metals in the environment; therefore, the use of effective monitoring techniques is essential to plan and execute evidence-based solutions to the problems related to these pollutants.

Keywords: Pesticides, Bioindicator, Chiroptera, Atlantic Forest, Theobroma cacao.

INTRODUÇÃO GERAL

As florestas tropicais sustentam aproximadamente dois terços da biodiversidade do mundo (RAVEN, 1988). A sua existência é essencial para a humanidade porque fornecem comida, água (ELLISON et al., 2012; FERRAZ et al., 2014), retêm nutrientes do solo (REID et al., 2005) e previnem o aquecimento global, pois podem capturar grande parte do carbono liberado pelo uso de combustíveis fósseis, ajudando a estabilizar as concentrações atmosféricas de CO_2 (PAN et al., 2011; HOUGHTON et al., 2015).

A conversão de espaços naturais em áreas urbanas e agrícolas promove a fragmentação e o desmatamento sistemáticos das florestas tropicais em todo o mundo, prejudicando seu funcionamento (SPRACKLEN et al., 2015). Nesses ambientes alterados, as comunidades naturais são frequentemente afetadas negativamente pelas atividades humanas que ali ocorrem (WILLIAMS-GUILLÉN et al., 2008b; PARK, 2015)

Além da perda direta de habitat causada pelas terras agrícolas, o uso excessivo de agroquímicos expõe os organismos a substâncias nocivas capazes de colocar em risco sua sobrevivência (CSIC; ROIG, 1996). Uma parte considerável dos produtos defensivos usados na agricultura não chega aos seus organismos alvo e permanece como resíduo no meio ambiente, o que pode afetar a biota nativa ou mesmo as populações humanas assentadas no entorno das plantações (NARANJO, 2014; PIGNATI et al., 2017). Esses produtos contêm contaminantes persistentes, como organoclorados e metais pesados, que podem se bioacumular nos tecidos e afetar várias funções vitais dos organismos, prejudicando-os, o que pode causar declínios significativos nas populações naturais (STAHLSCHMIDT; BRÜHL, 2012; STECHERT et al., 2014; ZUKAL et al., 2015).

Os compostos conhecidos como metais pesados são elementos metálicos e metaloides de densidade específica superior a 5g/cm^3 e que possuem alta capacidade de contaminação ao meio ambiente e aos organismos (HUAMAIN et al., 1999). Dentro deste grupo, os elementos arsênio, cádmio, cobalto, cromo, cobre, mercúrio, manganês, níquel, chumbo, estanho e tálio são considerados os mais potencialmente perigosos para a vida selvagem (BEYERSMANN; HARTWIG, 2008). Uma vez no ambiente, podem se bioacumular nos organismos, sendo absorvidos pela dieta, por contato direto ou por inalação (ALI; KHAN, 2019). Embora existam inúmeras evidências sobre os danos que estas substâncias podem ocasionar na saúde, além dos agroquímicos, diversas atividades antrópicas como a mineração, fabricação de vários produtos, queima de lixo ou combustão de gasolina, podem facilitar a entrada dos metais pesados nos ecossistemas. (MELANCON, 2002; LI et al., 2014).

A avaliação de contaminantes no ambiente pode ser facilitada estimando a sua bioacumulação em organismos capazes de refletir a poluição do ambiente em que habitam, como os quirópteros (ZUKAL et al., 2015; MANSOUR et al., 2016; MINA et al., 2019). Estes animais possuem características biológicas e ecológicas que nos permitem utilizá-los como bioindicadores sensíveis para evidenciar a presença de metais pesados e outros contaminantes no meio ambiente (RUSSO; JONES, 2015). Sua longevidade relativamente alta para um animal de seu tamanho, aumenta o tempo de exposição a potenciais contaminantes (GAISLER; CHYTIL, 2002). Possuem rápido metabolismo com altas taxas de ingestão de alimentos (KURTA et al., 1989) permitindo a incorporação de grandes quantidades de contaminantes através da dieta, que em muitos casos é composta por várias espécies de invertebrados que se desenvolvem em ambientes aquáticos, suscetíveis à entrada de poluentes de lavouras e fábricas devido ao escoamento da água (ZUKAL et al., 2015). Além de isso, muitos morcegos vivem em simpatria com as pessoas, habitando paisagens urbanas, industriais e agrícolas, podendo assim se expor a poluentes de diversas atividades humanas (HARIONO et al., 1993; RAMOS-H et al., 2020).

Diversos estudos realizados em morcegos que vivem em regiões sob a influência de metais pesados mostraram que são sensíveis a mudanças temporais ou espaciais nos níveis de poluição (RUSSO; JONES, 2015; ZUKAL et al., 2015). Um dos primeiros relatórios que envolveram metais pesados e morcegos selvagens foi realizado no Japão. Os autores fizeram uma comparação entre espécimes de museus e amostras recentes, durante e após o uso de fungicidas organomercuriais. Eles descobriram que houve um aumento do teor de mercúrio em amostras de morcegos coletadas durante e após o uso desses fungicidas (MIURA et al., 1978). Outro estudo apontou que houve uma correlação entre a concentração de chumbo encontrado nos ossos e pelos de morcegos e o grau de poluição do ar entre 1987 e 1999 (HARTMANN 2001). Em um estudo mais recente, as concentrações médias de mercúrio na pele de morcego foram correlacionadas com a variação espacial na contaminação atmosférica de mercúrio em mais de 40 locais espalhados por grande parte do Canadá (CHÉTELAT et al., 2018).

Por outra parte, numerosos estudos indicam que o conteúdo em pelo dos morcegos apresenta correlação com as concentrações dos metais pesados nos órgãos internos e no meio ambiente que habitam e por esse motivo, o pelo é amplamente utilizado para estimar de forma confiável e minimamente invasiva a bioacumulação (YATES et al., 2014; HERNOUT et al., 2016; MINA et al., 2019; TIMOFIEIEVA et al., 2021). Fatores tais como a espécie, idade ou o sexo podem condicionar a forma de exposição e/ou o metabolismo dos metais pesados nos morcegos e consequentemente ter incidência na bioacumulação (WALKER et al., 2007; ZUKAL et al., 2015)

Em geral, os morcegos frugívoros são primariamente expostos a metais pesados através da poluição atmosférica e secundariamente através do contato com folhagens contaminadas durante o forrageamento (HARIONO et al., 1993). Uma vez no pelo, os contaminantes podem ser rapidamente ingeridos durante a higiene (ZUKAL et al., 2015). Por outro lado, os morcegos insetívoros podem bioacumular metais presentes nas redes tróficas, alimentando-se de insetos que se desenvolvem em corpos d'água ou solo contaminados (HSU et al., 2006; HERNOUT et al., 2013). Embora não haja informações sobre as concentrações limites que causam efeitos adversos (ZUKAL et al., 2015), alguns estudos associam a intoxicação por metais pesados em morcegos a diversas patologias como doença hepática, danos ao DNA, hemocromatose, alterações de comportamento e funções colinérgicas, entre outras (SKERRATT et al., 1998; ZOCCHE et al., 2010; NAM et al., 2012; LEONE et al., 2016). Esses efeitos adversos podem agir sinergicamente com outros estressores, podendo causar o declínio das populações de morcegos em todo o mundo (ZUKAL et al., 2015) e afetando, e consequência, as funções chave que desempenham nos ecossistemas.

Várias espécies de morcegos contribuem com o reflorestamento de áreas degradadas e a manutenção da diversidade vegetal, devido ao seu papel como polinizadores e dispersores de sementes (KELM et al., 2008). Além disso, as espécies que são predadoras de insetos ou de pequenos vertebrados ocupam níveis superiores da cadeia trófica, exercendo controle top-down sobre suas presas e contribuindo assim para a manutenção do equilíbrio dos ecossistemas (MAAS et al., 2019; de SOUZA et al., 2020). Os serviços ecossistêmicos dos morcegos são essenciais para as florestas tropicais, portanto, a expansão agrícola e o consequente uso de agroquímicos colocam em risco ambos.

Em Brasil, a conversão para diferentes tipos de terras agrícolas levou à redução da Mata Atlântica a menos de 12,4 % de sua enorme extensão original de 1,36 milhão de km², (MYERS et al., 2000; SOS MATA ATLÂNTICA, 2019). Esta floresta tropical é um dos cinco principais hotspots de biodiversidade do nosso planeta, pelo que a sua conservação é prioritária, uma vez que alberga milhares de espécies, com uma elevada percentagem de endemismo em todos os táxons (MYERS et al., 2000; RIBEIRO et al., 2011). Abriga uma das maiores concentrações de diversidade arbórea do mundo, por exemplo, 454 espécies foram registradas na Bahia em um único hectare de floresta (THOMAS et al., 1998). Possui uma rica diversidade de tetrápodes composta por 517 espécies de répteis, 719 de anfíbios, 1.025 espécies de aves e 384 espécies de mamíferos, das quais 122 são morcegos distribuídos nas famílias Phyllostomidae, Noctilionidae, Emballonuridae, Natalidae, Thyropteridae, Vespertilionidae e Molossidae (BERGALLO et al., 2003; FIGUEIREDO et al., 2021). A maior parte das terras agrícolas que fragmentam e degradam esse bioma é manejada intensivamente com agroquímicos perigosos, muitos deles proibidos em outras partes do mundo, que ameaçam a vida selvagem e as pessoas (PIGNATI et al., 2017; SIQUEIRA, 2021). No entanto, ambientes agrícolas com baixa intensidade de manejo, como fazendas orgânicas ou agroflorestas sombreadas, podem reduzir os danos causados pelo impacto humano sobre as espécies que os habitam. (WILLIAMS-GUILLÉN et al., 2008; PARK, 2015).

Na região sul do Estado de Bahia, os sistemas agroflorestais de cacau combinam a produção agrícola com a conservação, pois levam apenas uma parte da mata nativa para ocorrer.

Esse sistema ambientalmente amigável representa terras fundamentais para garantir a manutenção da biodiversidade na região. Nesse contexto, os sistemas agroflorestais de cacau no sul da Bahia estão entre os poucos habitats modificados e amigáveis à biodiversidade, sabendo-se que abrigam grande número de espécies de vertebrados, incluindo uma abundante comunidade de morcegos (FARIA; BAUMGARTEN, 2007). Nesta região é tradicionalmente implementado o sistema agroflorestal sombreado chamado *cabruca*, que consiste no cultivo do cacau (*Theobroma cacao* L.) sob o sombreamento de árvores de maior tamanho, geralmente nativas. Esses sistemas agroflorestais, além de apresentar várias vantagens produtivas (como redução da erosão e perda de nutrientes do solo) são capazes de sustentar uma biodiversidade considerável, oferecendo refúgio a espécies de numerosos táxons, como borboletas, anfíbios, répteis, aves e mamíferos (FARIA et al., 2007). Também podem funcionar como corredores ecológicos, conectando remanescentes de florestas, permitindo que organismos transitem entre habitats e reduzindo o efeito de borda (SCHROTH; HARVEY, 2007). Junto com pequenos trechos de remanescentes de Mata Atlântica, esses sistemas agroflorestais fazem parte dos mais importantes refúgios de vida selvagem (PARDINI et al., 2009). No entanto, outras culturas presentes na região fazem uso excessivo de agroquímicos que podem colocar em risco a fauna que faz uso desses ambientes (DE SOUZA IVONETE, 2016). A toxicologia de morcegos no Brasil é pouco estudada e existem poucos estudos que avaliaram a presença de metais pesados nestes animais (de SOUZA et al., 2020).

É alarmante que o uso de metais pesados ainda seja difundido no mundo e que muitas atividades continuem a depositá-los no meio ambiente na década em que devemos restaurar, prevenir, frear e reverter a degradação dos ecossistemas, segundo o Nações Unidas (DIARRA; PRASAD, 2021; FISCHER et al., 2021). É vergonhoso que a gasolina com chumbo só tenha sido completamente abolida em 2021, um século depois seus efeitos nocivos eram conhecidos (PERCIVAL, 2020b), e é uma triste ironia que o manganês, outro metal pesado, seja usado atualmente como substituto (USTUN, 2022; HANNAH, 2022). Devido à falta de consideração pela ecologia com a qual a maioria das decisões são tomadas, nos perguntamos quando medidas adequadas serão tomadas para evitar danos irreparáveis à humanidade e ao meio ambiente. De forma alarmante, muitas pessoas no mundo vivem em áreas contaminadas por metais pesados (EDOGBO et al., 2020; MOHAMMADI et al., 2020). Na Floresta Amazônica, o problema negligenciado da mineração ilegal envenena os rios e solos em que as pessoas obtêm seus alimentos com mercúrio (VILLÉN-PÉREZ et al., 2022).

Apesar das muitas vias de entrada de metais nos ecossistemas, a avaliação de sua presença no ambiente é pouco abordada (de SOUZA et al., 2020). Planejar e executar soluções para problemas relacionados a metais pesados requer técnicas de monitoramento eficazes que permitam articular a tomada de decisão com as evidências (FLACHE et al., 2015, 2016; MOHAMMADI et al., 2020; SHIKHA; SINGH, 2021). Muitos desses contaminantes são comumente encontrados em paisagens agrícolas e sua presença pode ser avaliada por meio de biomonitoramento minimamente invasivo, como análise de amostras de pelo. O Brasil é o maior consumidor de agroquímicos do mundo (PIGNATI et al., 2017), portanto, trabalhos que abordem o problema com metais pesados devem ser realizados em todos os locais onde a vida selvagem e as pessoas possam estar em risco.

OBJETIVO GERAL

O presente trabalho teve como objetivo avaliar a bioacumulação de cobre (Cu), manganês (Mn) e chumbo (Pb) em morcegos de fazendas de cacau e como é afetada pela paisagem em diferentes contextos agrícolas.

OBJETIVOS ESPECÍFICOS

1) Avaliar a incidência dos principais componentes da paisagem na bioacumulação observada em morcegos.

2) Avaliar se o sexo ou a espécie dos morcegos têm efeito sobrea bioacumulação de metais pesados.

Dessa forma, testamos as seguintes hipóteses: (1) a ocorrência de matrizes agrícolas na paisagem pode aumentar a exposição de morcegos a metais pesados e consequentemente a bioacumulação; (2) O sexo e a espécie dos morcegos terá impacto na bioacumulação, já que diferenças fisiológicas e/ou comportamentais determinadas por estes fatores podem afetar a forma em que os organismos se expõem e/ou metabolizam os metais pesados.

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CHAPTER 1

Barillaro, J., Souza, A. P. de, **Bovendorp, R.S**. AGRICULTURAL LANDSCAPES AFFECTS LEAD MANGANESE AND COPPER BIOACCUMULATION IN BAT COMMUNITIES OF SHADED CACAO PLANTATIONS OF BAHIA STATE, NORTHEASTERN BRAZIL.

Manuscript formatted for submitted to **Ecotoxicology and Environmental Safety– Journal**. IF: **6.291** (2020)

Only the first and last author (highlighted in bold) have written and revised this version at the current moment. Other co-authors will make their contributions after the dissertation's defense.

To: Ecotoxicology and Environmental Safety– Journal

Title: Agricultural Landscapes Affects Lead, Manganese and Copper Bioaccumulation in Bat Communities of Shaded Cacao Plantations of Bahia State, Northeastern Brazil

Julián Barillaro [*,1], Adailson Pereira de Souza [2], Ricardo Siqueira Bovendorp [1]

[1] Programa de Pós-Graduação em Ecologia e Conservação da Biodiversidade, Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz – UESC, Ilhéus, BA, 45662-900, Brazil

[2] Programa de Pós-Graduação em Ciência do Solo, Centro de Ciências Agrárias, Universidade Federal da Paraíba, Areia, Paraíba, Brazil

[*] Corresponding author: Julián Barillaro (julian.barillaro@gmail.com)

Highlights

- Neither species nor sex of the individuals seem to have affected bioaccumulation.
- Landscapes with intensive agriculture had higher lead and manganese bioaccumulation.
- The bioaccumulation of lead reflected the anthropic advance on the forest

Abstract

Entire agricultural landscapes around the world are sprayed with bioaccumulative pollutants such as heavy metals, most of which do not reach their target organism, but remain in ecosystems to the detriment of their inhabitants. In Bahia, Brazil, an important agricultural product, cocoa (*Theobroma cacao*), is largely grown as structurally complex agroforestry alongside native species. It is considered a biodiversity-friendly land use, as supports considerable biodiversity, including a wide range of bats. However, the consequences of years of agrochemicals practices in the local crops are scarcely studied. To evaluate the bioaccumulation of copper, manganese and lead in bats of cacao farms and how this bioaccumulation is affected by landscape, we mist-netted bats and collected hair from postero-dorsal region for subsequent analysis of metal concentration by atomic absorption spectrometry. Also, we use generalized linear mixed models (GLMM) to assess the effects of local land uses on metal bioaccumulation. We found higher concentrations of lead (41.20 µg/g) and manganese (0.44 µg/g) in landscapes more strongly affected by deforestation and intensive agriculture than in landscapes with a greater presence of forest and shaded cocoa agroforestry. Likewise, cocoa plantations seem to promote the bioaccumulation of copper, most likely due to the use of copper fungicides in these crops. We have supported that heavy metals can be found in agricultural landscapes and their presence on the ecosystem can be evaluated through minimally invasive biomonitoring, such as analysis of hair samples. The results of this study may support the adoption of sustainable farming techniques such as shaded cacao agroforestry that minimize chemical deployment over the ecosystem.

Keywords: Agrochemicals; Atlantic Forest; Bioindicator; Chiroptera, Pollution.

Introduction

Tropical forests are increasingly being converted to agricultural lands, a process leading to a massive biological simplification due to species loss (Edwards et al., 2019). Additionally, most of the resulting farmlands are intensively managed, using chemical inputs to maximize production, but that are known to have pervasive effects on non-target organisms, with subsequent cascade consequences for the ecosystem (Oliveira et al., 2021). Several agrochemicals widely used contain heavy metals (Gimeno-Garcia et al., 1996), harmful pollutant with the ability of bioaccumulate in organism and threaten the viability of wildlife populations (Ali and Khan, 2019). The effects of bioaccumulation - the gradual accumulation of a certain chemical into the living tissue of an organism from its environment- of heavy metals are as numerous as they are dangerous. Consequences such as, hepatopathy, DNA and kidney damage, behavior changes, paralysis, tremors, spasms, hemochromatosis, general slowness, lack of control in body movement and, mortality are just some adverse effects reported for human and animals exposed to these chemicals' elements (Wolfe et al., 1998; Ali and Khan, 2019; Pain et al., 2019). Heavy metals threaten the survival of wildlife and are, in fact, a major cause of declining bat populations in various parts of the world (Zukal et al., 2015).

Various characteristics of bats make them susceptible to bioaccumulative pollutants from agrochemicals, such as organochlorines and heavy metals. They are relatively long-lived animals for their size, increasing not only the time available for bioaccumulation, also to transfer of lipophilic contaminants to offspring through milk (Zukal et al., 2015). Bats not only have high metabolic rates that demand a high daily intake of potentially contaminated food, but they are also often at relatively high trophic level. This makes them especially susceptible to biomagnified metals in the food chains (Yates et al., 2014; Hernout et al., 2016). Also, bats frequently habitat in agricultural landscapes, where they can be exposed to agrochemicals by direct contact (Oliveira et al., 2021), through contaminated food (Murugan et al., 2021), or by the contamination of surrounding water bodies (Naidoo et al., 2013). In this type of environment, other threats, such as loss of roost, habitat disturbance, and disease, can exert their detrimental effects in synergy with those of bioaccumulation of pollutants, putting bats in critical danger worldwide (Zukal et al., 2015). In fact, more than half of the threatened bat species are in conflicts related to agriculture (Frick et al., 2020).

Bat populations decline can be catastrophic for agriculture, since bats may increase the economic yield of crops through a top-down control of pest insects (Williams-Guillén et al., 2008; Boyles et al., 2011; Karp & Daily, 2014), and the pollination of many plants of economic interest, such as durian, mango, pitaya fruit, agave and jackfruit (Kunz et al., 2011; Parolin et al., 2016; Tremlett et al., 2020). Likewise, threats to bats can have serious consequences for the natural environment, since they pollinate at least 800 species of forest plants and play a preponderant role in dispersing the seeds of the species they feed on (Ramírez-Fráncel et al., 2022). Therefore, bats are a key group for the conservation of biodiversity, since they promote the restoration and diversity of forests, mainly in the Neotropics, where fruit-eating bats of the Phyllostomidae family are endemic (Trevelin et al., 2013).

In the southern region of the state of Bahia, Brazil, there is a close link between bats, forest conservation and agriculture (Faria et al., 2007; Schroth & Harvey, 2007, Cassano et al., 2009). Cocoa cultivation is one of the main agricultural activities in this region and constitutes an alternative for the economic sustenance of numerous families in rural areas (Cassano et al., 2009; Piasentin and Saito, 2014; Gama-Rodrigues et al., 2021). Moreover, this crop can contribute to the conservation of the Atlantic Forest since most of its production is carried out in agroforestry systems rich in species, together with native trees used to provide shade, which improves the properties of the crop and reduces the need of intensive management (Schroth and Harvey, 2007). Also, these crops can reduce the edge effect in remaining forests, serving as ecological corridors and refuge for numerous taxa, including more than 39 species of bats and some threatened species such as the golden-headed lion tamarin *Leontopithecus chrysomelas* and maned three-toed sloth *Bradypus torquatus* (Cassano et al., 2009).

However, metals such as lead, copper and cadmium have been found in the soils and trees of cocoa plantations in other parts of the world (Aikpokpodion et al., 2010; Arévalo-Gardini et al., 2017; Arham et al., 2017) and some agrochemicals that contain heavy metals are often used in the crops of the southern Bahia (de Oliveira & Luz, 2005; de Souza, 2016). Very few studies have evaluated the presence of these substances in the environment, finding copper in trees and soils of cocoa plantations (Franz-Gerstein, 2000) and zinc, lead, chrome and aluminum in the sediment of the Cachoeira river - over whose basin are many of the cacao plantations - some of these in concentrations several times higher downstream of urban and agricultural areas (Klumpp et al., 2002; Soares and Santos, 2013). In this way, the species that inhabit these cocoa agroforests could be exposed to hazardous substances that could put their livelihoods at risk. Nevertheless, the presence of heavy metals in wildlife of shaded cacao agroforestry of Southern Bahia has never been evaluated.

Recent studies have shown that heavy metal pollution can be assessed by analyzing hair of bio-monitor organisms, such as bats (Mina et al., 2019; Timofieieva et al., 2021a). The metal content in the hair correlates with that of various internal organs, which allows knowing through a minimally invasive analyze both, bioaccumulation in bats and environmental contamination of their habitat.

The objective of this study is to evaluate the bioaccumulation of copper (Cu), manganese (Mn) and lead (Pb) on bats of cacao farms and how this bioaccumulation is affected by landscape in an agricultural context. Agricultural lands can be a pathway for heavy metals to enter the ecosystem (Lima, 1994; Gimeno-Garcia et al., 1996; Nicholson et al., 2003; Peng et al., 2019) and previous studies found that the exposition of bats to pollutants may be affected by landscape composition (Stechert et al., 2014; Valdespino & Sosa, 2017; Ramos-H et al., 2020). Thus, we hypothesized that occurrence of agricultural land in the landscape can increase the exposure of bats to metals and consequently their bioaccumulation. This work aims to contribute to the scarce literature on the toxicology of bats in the Atlantic Forest and to the neglected problem of heavy metals in Brazil (de Souza et al., 2020; Vergilio et al., 2020), and in turn evaluate the contamination by these substances in an important ecosystem for conservation of Atlantic Forest.

Materials and methods

Study area

The study was carried out in 15 shaded cocoa plantations (*Theobroma cacao* L. – Sterculiaceae), and two Atlantic Forest remnants in southern Bahia, Brazil (Figure 1). This region belongs to Atlantic Forest biome, and is characterized by a humid tropical climate, with an average relative humidity of 80–90%, annual average temperature of 25 °C and annual precipitation of up to 2000 mm with no identified seasonality, although a rain-free period of one to three months can occur from December to March (Mori et al., 1983; Thomas et al., 1998). In addition to remnants of native vegetation, this region is occupied by shaded cocoa plantations, eucalyptus agroforestry, pastures, rubber and mangroves (Pardini et al., 2009; Tabarelli et al., 2010).

The shaded cocoa plantations sampled are located in rural areas of the municipalities of Ilhéus (14° 39´ 40" S, 39° 11´ W), Belmonte (15° 50´ S, 39° 23´ W) and Una (15° 12´ S, 39° 10´W, Figure 1). The predominant crops and the degree of conservation of the forest remnants differ in these three regions so that we can define three different landscapes (Faria et al., 2021; Ramos et al., 2022). In Ilhéus, it is mostly made up of large farms of shaded cocoa (LFSC) agroforestry managed by large producers, in addition to some patches of forest remnants. In Belmonte, cocoa occupies a smaller place and the landscape is dominated by pastures and monocultures (PM) with greater intensity of management, such as eucalyptus forestry. Likewise, the native forest is highly degraded and there are few remnants (Pessoa et al., 2017). On the other hand, in the Una region, the landscape is made up of small farms of shaded cocoa (SFSC) agroforestry, associated with banana, açaí and other fruit crops, generally small and managed by minor owners and local families. In addition, this region is better preserved and has larger forest remnants than Ilhéus and Belmonte (Morante-Filho et al., 2016). In fact, it has two merged protected areas, the Una Wildlife Refuge and the Una Biological Reserve (15° 10´ S, 39° 6´ W). Its more than 40,000 hectares are part of the Central Atlantic Forest Ecological Corridor. Since they are one of the largest and best-preserved native forest remnants within the study area, these were sampled as control sites (Figure 1). Hereinafter, for practicality, we will refer to these as a whole, calling it Una Biological Reserve (UBR).

Bat sampling

Bats were sampled between February to August 2021, using six mist nets of 2.5 m height, strategically placed in possible bat flight paths. The nets remained open for four hours from sunset and were inspected at intervals of approximately 15 or 30 minutes depending on capture rate. After captured, bats were held in individual cotton bags, identified to species level, processed and released in the same place where they were captured. Hair was carefully clipped from the mid-dorsal region in an area of approximately 1 cm^2, as close to the skin as possible, with aid of previously ethanol cleaned steel scissors to avoid cross contamination (Mina et al., 2019). Hair samples were conditioned in contamination-free polyethylene bags and stored at -20 °C until chemicals analyses. As the hair remotion allows the identification of recaptures for a considerable period (Lawton and Stonehouse, 1980) and, in addition, diverse studies with Neotropical bats have shown low recapture rates (Bernard & Fenton, 2003; Racey, 2011) we decided not to use any additional kind of marks, in order to minimize handling time and unnecessary stress for the bats.

Only adult bats were used in this study and they were identified through the epiphyseal-diaphyseal fusion, watching through the wing membrane using a headlight (Wilkinson, 2009). The current work followed Zukal, 2020 taxonomic nomenclature (Zukal, 2020). The captured bats were managed under authorizations by SISBIO / ICMBio:7672-1 and CEUA/UESC: 008/21.

Chemical analyses

The samples were weighed and subsequently digested with nitric acid (HNO_3) – perchloric acid ($HClO_4$) digestion. Initially, 6 ml of nitric acid were added to the samples, which were left to rest for approximately 12 hours. After 12 hours, complete digestion was started. The samples were heated at a temperature of 80-90 °C for 1~2 hours and after this period, they were gradually heated until reaching 120 °C for to be volume reduced. After that, 1 ml of perchloric acid was added and the temperature was gradually increased to 180 °C. Then, when white smoke of $HCLO_4$ formed and the sample extracts were translucent, we added deionized water to stop the digestion. Later, once the samples had cooled, they were brought to a volume of 20 ml with ultrapure water. Concentrations of manganese (Mn), lead (Pb) and copper (Cu) were measured using Atomic Absorption Spectrometry 240FS AA.

Statistical analyses

Differences in metal concentrations between species, sexes and sampling sites.

As we sampled in 4 different study sites, differences in metals concentration between species and between sexes within species were evaluated considering the landscape type (LFSC, PM, SFSC, UBR) as a factor. Since the data did not have a normal distribution (Shapiro-Wilk tests), non-parametric statistical tests were used throughout the study. When Kruskal-Wallis test was significant, differences between groups were analyzed with Dunn's, 1964 multiple pairwise comparisons as post hoc method.

Landscape composition characterization

To assess the effect of landscape composition on the bioaccumulation of the analyzed metals, the landscape surrounding the sampling sites was characterized in order to obtain the explanatory variables that would later be used to build statistic models. For this, it was used a land use raster of Coverage and Land Use Maps in Brazil year 2020 by MapBiomas Project and QGIS (QGIS 3.22.3-Białowieża, Python version 3.9.5). We estimated the landscape composition in sets of buffers of 1,000 to 5,000 m radius every 500 m at sampling sites. The land use represented by the raster map were forest, shaded cacao agroforestry, water bodies, urban areas, open areas, wetlands and eucalyptus agroforestry (Figure 1).

Modeling effect of landscape components on metal concentration

In order to avoid biases in coefficient estimates of the models, every set of buffers size were checked for collinearity between landscape variables (r >|0.60|). Additionally, because some of the larger buffers showed a slight overlap between nearby sites, we checked the spatial autocorrelation in the landscape variables using Moran's I autocorrelation Index (Kot, 1990). As a result, landscape data of buffers bigger than 2000 m were discarded. Therefore, we fit models considering only bat species with sedentary habits that do not change roost frequently (Soriano, 2000). In this subgroup we consider the species *Carollia brevicauda* (Schinz, 1821), *Carollia perspicillata* (Linnaeus, 1758), *Gardnerycteris crenulatum* (E. Geoffroy, 1803), *Glossophaga soricina* (Pallas, 1766), *Glyphonycteris sylvestris* (Thomas, 1896), *Phyllostomus discolor* (Wagner, 1843), *Phyllostomus hastatus* (Pallas, 1767), *Rhinophylla pumilio (*Peters, 1865), *Sturnira lilium* (E. Geoffroy, 1810), *Tonatia bidens* (Spix, 1823), *Trachops cirrhosus* (Spix, 1823) and *Micronycteris megalotis* (Gray, 1842). We assume that these species, which in general have a smaller home range and space use than species with nomadic habits (Rhodes, 2007; Carter et al., 2010; Trevelin et al., 2013), would more likely represent the effect of the landscape on the scale of available buffers. Also, the landscape variables “urban areas” and “water bodies” were zero in most cases. For this reason, they were not considered in the models in order to avoid convergence problems.

Then, we tested the effects of forest, open areas, wetlands, eucalyptus and shaded cacao plantations covariates in the hair concentration of every metal analyzed using generalized linear mixed-effects models (GLMMs) with the package lme4 (Bates et al., 2014). We considered sample site as random effects in all models, coding them as (1|Site).

Model selection

The models fitted with GLMM were zero inflated, then, we used Zero-Inflated Gamma Mixed Models (ZIGMM) of the R package glmmTMB (Brooks et al., 2017) to fit models containing all landscape explanatory variables at each buffer size. Later, we compared the global models Akaike information criterion (AICs) to select the buffer size that produced the best fit and then, we used the landscape variables at that size to build all possible models. Finally, we compared the models sets using second-order AIC (AICc) and weights (w) and then, we constructed the averaged model (Burnham and Anderson, 2002; Anderson, 2008). Due to the size of our dataset, we built single-variable models, restricting the number of candidate models to six. Model selection process was performed using the R package “MuMIn” (Barton & Barton, 2020). All statistical analyses were performed in the software R (R Core Team, 2021).

Results

Differences in metal concentrations between species, sexes and sampling sites

We obtained a total of 326 fur samples from 28 species of bats, the majority of the family Phyllostomidae. We found Mn, Pb or Cu in 80% of them. Pb was the most common, followed by Mn and Cu being found in 68, 20, and 10 percent of the samples, respectively. *Carollia perspicillata* was the most frequently sampled species with more of the 40% of the captures followed by *Artibeus obscurus* (Schinz, 1821), *Artibeus planirostris* (Spix, 1823), *Artibeus cinereus* (Gervais, 1856), *Rhinophylla pumilio* and *Glossophaga soricina*, each accounting for 5 ~ 9 percent of the total captures. These species were sampled in similar proportions at the different sites, except for *A. obscurus* and *A. planirostris*, which were not captured in SFSC nor UBR. Mean metal values and sample size for species at the different sites are shown in Table 1. No significant differences between species or sexes within species were observed for any metals. Then, values were pooled to compare Mn, Pb and Cu at different study sites (Table 2, Figure 2).

Hair Pb and Mn concentration differ between study sites ($X^2 = 29.242$, $p < 0.01$; $X^2 = 84.85$, $p < 0.01$). Samples from UBR had significantly lower Pb concentration than those of any other sites (all $p < 0.05$) and lower Mn concentration than PM ($z = 5.52$, $p < 0.01$). Furthermore, no sample from UBR had Cu. However, this metal was commonly scarce in all samples, showing no statistical difference between study sites ($X^2 = 2.57$, $p = 0.46$). On the other hand, PM had significantly greater Mn and Pb concentration than those of LFSC ($z = 8.25$, $p < 0.01$, $z = 3.08$, $p < 0.01$ respectively) and SFSC ($z = 7.5$, $p < 0.01$, $z = 2.14$, $p < 0.05$ respectively, Table 2, Figure 2). Finally, bats from LFSC and SFSC were not statistical different for any analyzed metals (all $p > 0.05$).

Landscape components affecting metals bioaccumulation

The global models for each metal had similar AICs at different buffer sizes. However, the global models for Mn, Pb and Cu had the lowest AICs at the 1000 m, 2000 m and 1500 m buffer sizes respectively.

Exploring the models generated for manganese, none was better than the null model (Table 3). The explanatory variables open areas and eucalyptus had positive coefficients, while cocoa agroforestry, forests and wetlands, had negative values. However, none of them stood out in importance or were significant in the averaged model (Table 4)

For lead, on the other hand, four of the six models generated were better than the null model (Table 3). The most important variable affecting lead bioaccumulation was forest coverage, with a negative effect, (Figure 3) followed by open areas coverage, with a positive and lesser effect. These two variables were the only informative in the averaged model (i.e. confidence intervals do not contain 0) and its relative importance was 0.390 and 0.240 respectively (Table 4).

In the case of copper, out of the six models generated, three were better than the null model. However, the best model ranked according to its AICc had a much higher weight than the others (Table 3). Consequently, we chose this model instead of generating an averaged one. The explanatory variable of this model was percentage of coverage of cocoa agroforestry, with a relative importance of 0.878 (Table 4), showing a positive effect on the concentration of Cu (Figure 3).

Discussion

To our knowledge, this is the first study to address heavy metal exposure in bats of cacao agroforestry systems in Brazil, as well as being the first record of Mn, Pb, and Cu values for many species of bats (Zukal et al., 2015; de Souza et al., 2020). Through our innovative approach, we found possible relationships between the composition of agricultural landscapes and the bioaccumulation of heavy metals. Neither species nor sex of the individuals seem to have affected bioaccumulation because we found no significant differences in any of those variables. Instead, when pooling data across species and sexes, landscape composition seemed to influence bioaccumulation. We found higher concentrations of Mn and Pb in the landscape dominated by intensive agriculture, and also the percentage of coverage of some land uses were important in the Pb and Cu models.

It is possible that the higher Pb and Mn concentrations found in PM (Belmonte) are related to the type of activities that take place in that region, particularly monocultures such as eucalyptus and coffee, usually intensively managed with agrochemicals. For example, certain fertilizers, such as iron sulfate, sometimes contain considerable amounts of Pb (Gimeno-Garcia et al., 1996). Furthermore, Pb was used for a long time as an additive in fuels, and due to its high persistence in the environment, consequences are still noticeable (Falta et al., 2005; Walker et al., 2007; Flache et al., 2015; Mielke et al., 2019). Since the use of fossil fuels is involved in most human activities, Pb pollution is associate with anthropization in general (Falta et al., 2005; Obeng-Gyasi, 2019; v. Percival, 2020). However, the eucalyptus cultivation carried out in PM promotes both the use of agrochemicals and the consumption of fuels. This industry is so intense that it is able to operate 24 hours a day. In order to get rid of weeds and ants, companies apply large amounts of agrochemicals, often undeclared, and there are numerous complaints of overuse by local communities (de Souza 2016). In addition, the felling of trees, preparation and transport of wood implies the use of heavy machinery with high fuel consumption (Guerra et al., 2016). In this way, this activity could facilitate the entry of Pb into the environment, consequently reaching the bats. Nevertheless, in the averaged model for Pb we not found that eucalyptus had a significant effect on its concentration. Instead, our results point that open areas and forests explain Pb concentrations. Although eucalyptus plantations could be a source of Pb, it is possible that at the local scale of the landscape considered by the model, a mainly atmospheric contaminant such as Pb cannot be directly related to a non-point source such as a crop. Rather, what the model seems to suggest is that Pb contamination is related to more open areas and less forest. The reduction of natural habitats could force organisms to use anthropized environments where they can be exposed to pollutants generated by human activities (Zukal et al., 2015). In this way, landscapes made up of large areas of intensive agriculture and pastures can increase the exposure of bats to pollutants and cause greater bioaccumulation of Pb. In agreement with these observations, the samples of SFSC (Una) and LFSC (Ilhéus), sites with a greater amount of forest than PM, had intermediate values of Pb while those from forest fragments of UBR had the lowest. There appears to be a relationship between deforestation and Pb bioaccumulation, both at regional and local scales, where Pb bioaccumulation appears to reflect agricultural expansion above the forest at both levels.

The predominance of intensive agriculture in PM may have increased the bioaccumulation of Mn. Some fungicides usually used in the region, such as Maneb ($C_4H_6MnN_2S_4$) and Mancozeb ($C_8H_{12}MnN_4S_8Zn$), contain this metal and can be a pathway for it to enter the ecosystem (Gimeno-Garcia et al. 1996, Oliveira and Luz 2005, de Souza 2016). However, none of the agricultural land uses we evaluated had a significant effect on the concentration of Mn.

Although Mn could come from other crops that we do not consider locally, such as coffee, it is possible that other activities such as mining may be involved in the entry of this pollutant into ecosystems. The removal of the substrate that is carried out in mining can release the metals present in the sediments into the environment (Röllin, 2017). In fact, large mining-industrial companies are increasing their activity in Santa María Eterna, a town belonging to the PM region (BMS Team, 2021). Thus, it is possible that the occurrence of these disturbances in the landscape increases the exposure of bats to Mn, influencing its bioaccumulation.

Cocoa agroforestry seem to have a lower impact on Pb and Mn bioaccumulation, since we found lower concentrations in SFSC and LFSC than PM for both metals, and similar to those found in UBR for Mn. Most of the cocoa produced in these regions is cultivated in structurally complex agroforestry systems, alongside native forest species. These ecosystems preserve various aspects of a true forest that reduce the need for the use of agrochemicals, as they maintain populations of natural predators and prevent the loss of nutrients from the soil (Novais et al., 2017; Mortimer et al., 2018). In addition, cocoa is planted and harvested manually, minimizing the use of machinery that could release Pb into the environment from fuel consumption (Sambuichi et al., 2012). Also, agroforestry system can inhibit the transport of toxic substances, minimizing environmental impact caused by pesticides (Pavlidis and Tsihrintzis, 2017). Therefore, these types of crops could reduce the exposure of bats to Pb and Mn. As we found similar metal values in SFSC and LFSC, it seems that both large and small cacao producers make reduced use of agrochemicals and their implementation is not conditioned by application costs.

The landscape composition affected the bioaccumulation of Cu. Although we did not find differences between the different sites, the local percentage of cocoa cover was important in the model. The coefficient and effect of this variable suggest that cocoa farms have a promoting effect of Cu bioaccumulation. The presence of Cu in our samples may be due to the use of fertilizers, manure, sewage water, and agrochemicals (Kabata-Pendias and Mukherjee, 2007). Cuprous oxide is usually used in the control of witches' broom (Oliveira and Luz, 2005), a fungal disease that strongly affected Bahia cocoa production in the early 1990s (Sousa Filho et al., 2021). In fact, some studies found considerable amounts of Cu in trees and soils of cocoa plantations where copper-based fungicides were applied (Franz-Gerstein, 2000; Lima, 1994). In this way, the relationship observed between this metal and cacao could be due to the current or past use of these kinds of agrochemicals.

This work covered a wide area in a region of high priority for conservation subjected to anthropic pressure (Pardini et al., 2009). The great variation that we found in our data may have contributed to masking possible differences in bioaccumulation due the species or sexes. In previous works, metal concentrations varied widely according to the species, sexes and location (Zukal et al., 2015), being the latter factor the one that seems to have prevailed in our study. In this way, although we expected to have found some differences due to the large number of species we captured, it is not surprising if we take into account that these factors affect bioaccumulation without a defined pattern and are not always determining factors (Zukal et al., 2015). Otherwise, the capture method used, mist nets in understory, allowed us to capture numerous species of frugivores, although it left other guilds such as insectivores underrepresented.

Pb values in our samples were much higher than found in Vespertilionidae and Molossidae bats from urban areas in Europe and mining areas of South Africa (Flache et al., 2015; Mina et al., 2019; Timofieieva et al., 2021b; Cory-Toussaint et al., 2022). The date on which leaded gasoline was phased out in each of the countries may have had an influence on these differences. Furthermore, unlike our work, these did not consider the metal attached to the hair surface, which may be important in contaminants where atmospheric exposure is elevated, such as Pb. A unique study performed in mining areas evaluated heavy metals in bats in Brazil (Zocche et al. 2010). Compared to our work, they had a pattern similar to the previous ones. However, they analyzed liver samples, which have a lower correlation with Pb than hair (Mina et al., 2019; Timofieieva et al., 2021b). In addition, our mean Cu values were lower than in those works, although with similar maximums, unlike Mn, which in our case was always lower. In these studies, bioaccumulation also seemed to be affected by anthropogenic activities. Finally, our results extend what was observed by Fritsch et al. (2011, 2012) in birds and invertebrates, where the composition of the landscape seemed to affect the exposure to metals. Also, in a more recent work (Valdespino and Sosa, 2017), concentrations of organochlorine compounds, other dangerous and bioaccumulative residues of the agrochemicals, were higher in bats from a human disturbed landscape than bats from a forested one.

Through this study, we have described relationships between anthropic modified landscape and the bioaccumulation of hazardous metals. Pb was the most abundant and frequent metal and was present in all study sites. It was twice as high in the landscape dominated by pastures and monocultures than in the one dominated by shaded cocoa agroforests, and more than ten times higher than in the control (UBR). In addition, it seemed to correlate with the anthropic advance on the natural remnants. Although leaded gasoline gradually began to be phased out from the 1970s onwards (Percival, 2020a), we verified that this contaminant still persists in the environment and, like many others, can be detected through bat hair analysis. Bone lesions and falls during fledging was associated to bats exposed to high doses of lead (Andreani et al., 2019). In this way, the bioaccumulation of lead found in this study can be a serious threat to bat communities of Southern Bahia, particularly in landscapes with a greater presence of intensive agriculture. Also, in this type of landscape, manganese values were more than five times higher than in those with a greater presence of shaded cocoa agroforest, and more than 50 times higher than in the control. Therefore, we encourage expanding the evaluation of the presence of manganese and other metals in Belmonte, also considering potential sources such as other crops and mining. In addition, we suggest considering landfills, since we noticed during the sampling campaign that open burning of garbage seemed to be a frequent practice in this region. This practice is detrimental to the environment and can release significant amounts of many metals (Karar et al., 2006). Moreover, although our mean copper values were generally lower than in other studies, there seems to be a relationship between the bioaccumulation of this metal and cocoa plantations. Due to the important presence of this crop in the region and that our maximum Cu values were sometimes higher than in other works (Flache et al., 2015; Mina et al., 2019; Timofieieva et al., 2021b; Cory-Toussaint et al., 2022;), the presence of this metal should not be overlooked.

Cocoa agroforestry play a fundamental role in the conservation of biodiversity (Cassano et al., 2009; Maney et al., 2022) and the numerous species that find refuge in these habitats could be exposed to this dangerous substance. In the natural environment, stressors, like heavy metals exposition, can exert their harmful effects synergistically with others, such as parasites (Marcogliese and Pietrock, 2011). This makes it difficult to know the threshold at which the metals we detect can be dangerous to wildlife (Zukal et al., 2015). All of them are hazardous and could threaten bat populations (Ljung and Vahter, 2007: Koz, 2010; Zukal et al., 2015). In this way, the ecosystem services they provide can be dramatically affected with dire consequences for the conservation of tropical forests.

Conclusions

In summary, we found that bioaccumulation of manganese and lead was higher in the Belmonte region, where the landscape is dominated by eucalyptus plantations and pastures over native forest and shaded cocoa agroforestry. The latter seems to have little impact on the bioaccumulation of these two metals. Likewise, the landscape did not affect the bioaccumulation of copper at a regional scale, since we did not find differences between the sites. However, the GLMM models associated it with the local percentage cover of cocoa plantations, probably due to the control of cocoa pests through the use of cupric fungicides. While manganese and copper were relatively scarce in our samples, lead was abundant, and also, it seemed to correlate with the anthropic advance on the natural remnants.

Cocoa plantations and bats in Southern Bahia are exposed to dangerous metals, most likely of anthropogenic origin. Intensive agriculture and forest reduction may promote greater bioaccumulation of lead and manganese. We support that shaded cocoa agroforests are friendly production systems, but that they can also promote copper pollution. Heavy metals are dangerous and persistent pollutants, capable of threatening species with key functions for ecosystems, such as bats. So, the results of this study may support the adoption of organic farming techniques that minimize chemical release to the ecosystem (Medeiros et al., 2010). The implementation of environmentally friendly production techniques is an essential requirement for any conservation program.

Acknowledgments/ Permits

We would like to thank Rebeca Sampaio, Paloma Resende, Maycon Santos, Letícia Costa, Franger García, Paola de la Quintana and Wilson Corea for their help in the fieldwork; and local landowners for allowing us to work on their property. We are grateful to the members of the Applied Ecology & Conservation Lab for their scientific and field contributions. We also thank the members of the Plant Tissue Analysis Laboratory at UFPB – Areia / Pb, for their aid in the hair analysis. The captured animals were managed under authorizations by SISBIO / ICMBio: 7672-1 and CEUA – UESC: 008/2021.

Financial support

The CNPq master scholarship awarded to J. Barillaro. Logistic facilities during the fieldwork were provided by The Applied Ecology & Conservation Laboratory – LEAC / UESC. This study is also part of the Instituto Nacional de Ciência e Tecnologia em Estudos Interdisciplinares e Transdisciplinares em Ecologia e Evolução – INCT INTREE (CNPq-proc. n. 465767/2014-1, CAPES-proc. n. 23038.000776/2017-54) and to Economia das Cabrucas Project.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables and Figures

Table 1

Species	Study site	Manganese (μg/g)			Lead (μg/g)			Copper (μg/g)			n
		Mean	SD	Max.	Mean	SD	Max.	Mean	SD	Max.	
A. caudifer	Ios	0	0	0	7.106	3.760	9.765	0	0	0	2
A. cinereus	Ios	0.124	0.248	0.671	31.868	26.287	61.021	1.781	3.758	10.497	8
	Una	0	0	0	28.808	16.212	50.567	14.207	40.182	113.653	8
	Bm	0.380	0.541	1.147	43.913	21.931	74.304	0	0	0	4
	Fr	0	0	0	1.275	1.762	4.286	0	0	0	5
A. fimbriatus	Ios	0	0	0	3.247	4.117	9.473	0	0	0	5
	Bm	0.655	0.747	1.437	41.612	36.888	78.232	0	0	0	4
A. lituratus	Ios	0	0	0	16.668	23.572	33.336	0	0	0	2
	Una	0	0	0	20.701	24.607	48.558	0	0	0	4
	Bm	0.574	0.791	1.720	56.024	41.981	123.720	0.067	0.163	0.400	6
A. obscurus	Ios	0	0	0	25.598	20.140	63.068	0.314	1.330	5.645	18
	Bm	0.582	1.164	2.328	22.741	21.625	54.516	0.367	0.734	1.469	4
	Fr	0	0	0	2.064	2.761	6.085	0	0	0	5
A. planirrostris	Ios	0.144	0.441	1.800	17.556	22.600	80.305	1.410	4.413	18.117	17
	Bm	0.200	0.377	1.065	9.876	9.920	22.245	0	0	0	11
C. auritus	Una	0	-	0	0	-	0	0	-	0	1
C. brevicauda	Bm	0.421	0.365	0.638	18.420	27.301	49.786	0	0	0	3
C. perspicillata	Ios	0.095	0.479	4.085	21.102	21.586	93.363	0.390	2.474	21.050	86
	Una	0.040	0.185	0.961	19.246	16.591	54.696	0.128	0.517	2.573	27
	Bm	0.808	0.891	3.100	48.334	49.330	399.750	0.309	0.629	2.412	22
	Fr	0	0	0	4.534	3.527	11.148	0	0	0	7
C. villosum	Ios	0	-	0	4.512	-	4.512	0	-	0	1
	Una	0	-	0	26.229	-	26.229	0	-	0	1
D. rotundus	Bm	0	-	0	83.270	-	83.270	0	-	0	1
	Fr	0	-	0	3.679	-	3.679	0	-	0	1
G. crenolatum	Bm	0	-	0	27.018	-	27.018	0	-	0	1
G. soricina	Ios	0	0	0	23.826	26.750	77.845	1.633	4.620	13.067	8
	Una	0	0	0	39.777	21.400	70.364	0	0	0	4
	Bm	0.467	0.279	0.664	13.870	19.615	27.739	0	0	0	2
	Fr	0	0	0	0	0	0	0	0	0	2
G. sylvestris	Una	0	-	0	66.765	-	66.765	0	-	0	1
M. megalotis	Bm	0.295	-	0.295	54.852	-	54.852	0	-	0	1
M. nigricans	Bm	0	0	0	71.772	27.768	103.182	0	0	0	3
P. discolor	Bm	0	0	0	18.749	5.874	22.902	0	0	0	2
P. hastatus	Ios	0	-	0	0	-	0	0	-	0	1
	Bm	0	-	0	14.299	-	14.299	0	-	0	1
P. helleri	Una	0	-	0	46.469	-	46.469	86.122	-	86.122	1
P. lineatus	Ios	0	0	0	0	0	0	0	0	0	2
	Bm	0.034	-	0.034	21.854	-	21.854	0	-	0	1
R. naso	Bm	0	-	0	19.938	-	19.938	0	-	0	1
R. pumilio	Ios	0.158	0.258	0.623	36.321	28.697	93.048	5.525	17.472	55.252	10
	Una	0	0	0	0	0	0	0	0	0	2
	Bm	0.232	0.249	0.496	19.753	17.156	30.933	0	0	0	3
	Fr	0.064	0.111	0.192	5.098	8.831	15.295	0	0	0	3
S. canescens	Bm	0	-	0	38.280	-	38.280	0	-	0	1
S. leptura	Ios	0	-	0	2.856	-	2.856	0	-	0	1
S. lilium	Ios	0	0	0	7.880	11.143	15.759	0	0	0	2
	Una	0	-	0	0	-	0	0	-	0	1
	Bm	0.082	0.142	0.247	44.999	29.900	75.295	0	0	0	3
T. bidens	Una	0	0	0	37.486	7.921	43.087	0	0	0	2
T. cirrhosus	Ios	0	0	0	3.622	3.476	7.370	0	0	0	4
U. bilobatum	Ios	0	0	0	21.907	23.595	51.185	10.448	23.362	52.240	5
	Una	0	0	0	38.732	13.150	52.612	0	0	0	3
	Bm	0.014	-	0.014	0	-	0	0	-	0	1

Table 2

Study site	Mn(μg/g) Mean	Mn(μg/g) SD	Mn(μg/g) Max.	Pb(μg/g) Mean	Pb(μg/g) SD	Pb(μg/g) Max.	Cu(μg/g) Mean	Cu(μg/g) SD	Cu(μg/g) Max.	n
Bm	0.445^{a}	0.690	3.100	41.207^{a}	55.815	399.75	0.116 ^{a}	0.398	2.412	75
Ios	0.077^{b}	0.376	4.085	20.893^{b}	22.213	93.363	1.151 ^{a}	6.287	55.252	172
Una	0.019^{b}	0.129	0.961	20.619^{b}	17.195	70.364	3.629 ^{a}	18.879	113.653	56
UBR	0.008^{b}	0.040	0.192	2.931^{c}	3.936	15.295	0 ^{a}	0	0	23

Table 3

	Intercept	Open areas	Wetlands	Cocoa	Eucalyptus	Forest	df	logLik	AICc	ΔAICc	Weight
Manganese	-0.2671						4	-133.678	275.6	0	0.281
	-0.5474	0.7677					5	-133.143	276.6	1.03	0.167
	-0.3238				3.709		5	-133.181	276.7	1.11	0.161
	0.1235			-1.013			5	-133.241	276.8	1.23	0.152
	-0.1237					-0.8095	5	-133.351	277	1.45	0.136
	-0.19		-1.526				5	-133.628	277.6	2	0.103
Lead	3.773					-1.74	5	-759.576	1529.5	0	0.393
	2.906	1.391					5	-760.083	1530.5	1.01	0.237
	3.216				7.96		5	-760.644	1531.6	2.14	0.135
	2.964		12.96				5	-760.868	1532	2.58	0.108
	3.333						4	-762.051	1532.3	2.85	0.095
	3.363			-0.08152			5	-762.048	1534.4	4.94	0.033
Copper	-0.9995			5.537			5	-92.785	195.9	0	0.878
	3.053	-4.084					5	-95.495	201.3	5.42	0.058
	3.635		-60.18				5	-96.461	203.2	7.35	0.022
	1.341						4	-97.607	203.4	7.54	0.02
	1.771				-36.21		5	-96.919	204.1	8.27	0.014
	1.332					0.05336	5	-97.607	205.5	9.64	0.007

Table 4

	Variable	Estimate	CI LL	CI UL	*z* value	*p* value	Importance
Manganese	Intercept	-0.236	-0.921	0.448	0.349	0.677	
	Forest	-0.810	-2.792	1.173	0.800	0.423	0.140
	Open areas	0.768	-0.778	2.314	0.789	0.973	0.170
	Eucalyptus	3.709	-4.283	11.701	4.078	0.910	0.160
	Cacao	-1.013	-3.104	1.078	1.067	0.949	0.150
	Wetlands	-1.526	-10.751	7.699	0.324	0.746	0.100
Lead	Intercept	3.350	2.491	4.210	7.638	0	
	Forest	-1.740	-3.166	-0.314	2.392	**0.017***	0.390
	Open areas	1.391	0.103	2.678	2.116	**0.034***	0.240
	Eucalyptus	7.960	-0.966	16.886	1.748	0.081	0.130
	Cacao	-0.082	-1.989	1.826	0.084	0.933	0.030
	Wetlands	12.960	-3.127	29.047	1.579	0.114	0.110
Copper	Intercept	-1.000	-2.247	0.248	-1.571	0.116	
	Cocoa	5.537	2.876	8.198	4.078	**0.000***	0.878

Figure 1

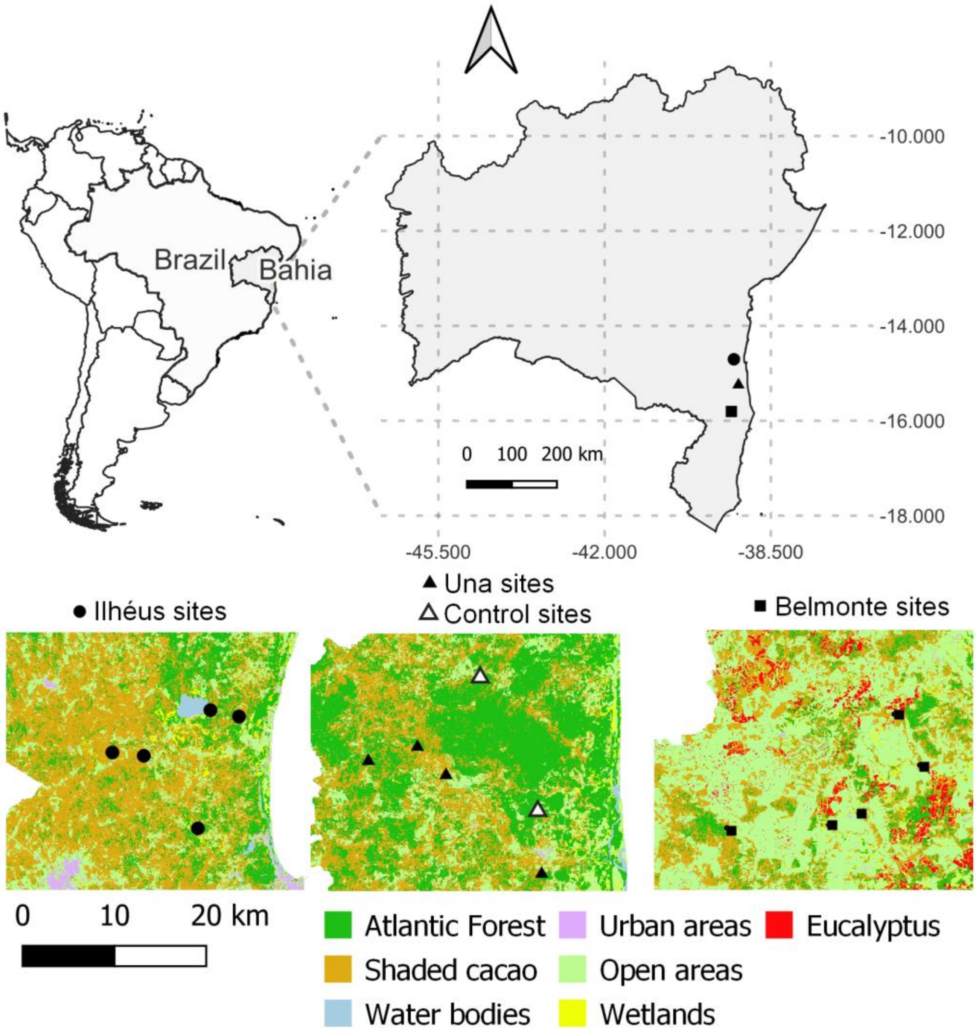


Figure 2

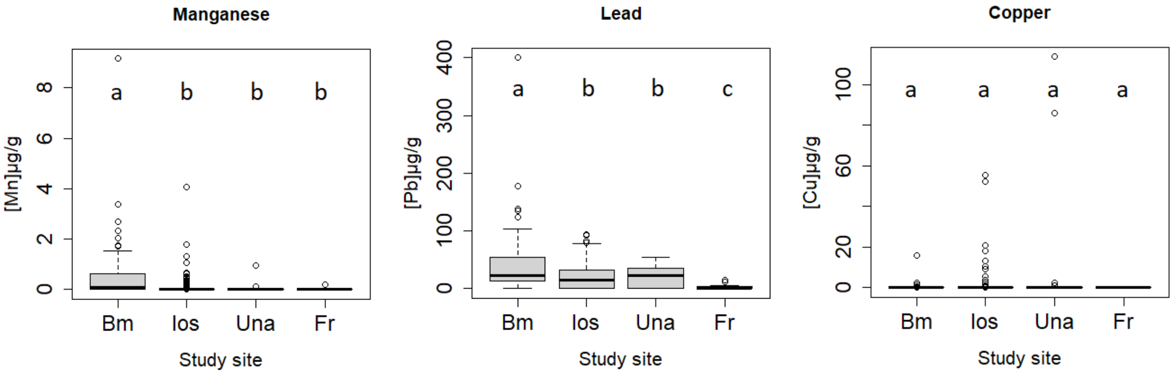
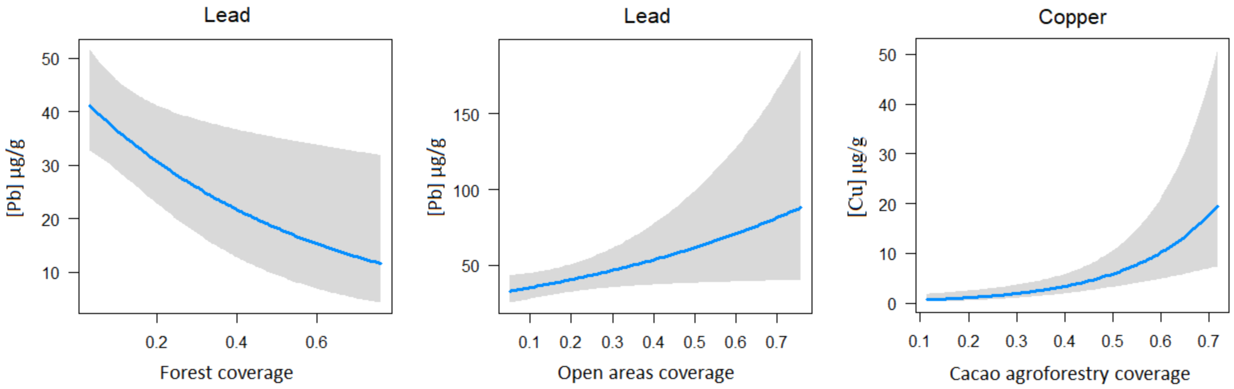


Figure 3



Tables and Figure`s caption

Table 1: Mean fur metal concentration, standard deviation (SD) and max value found in all bats species sampled in shaded cacao agroforestry sites of Ilhéus (Ios), Una, Belmonte (Bm) or forest sites within Una Biological Reserve (Fr) from southern region of the state of Bahia, Brazil.

Table 2: Mean hair metal concentration of bats from shaded cacao agroforestry sites in the different landscape context of Belmonte (Bm, Pastures and Monocultures, PM), Ilhéus (Ios, Large Farms of Shaded Cacao, LFSC), Una (Small Farms of Shaded Cacao, SFSC) and forest from Una Biological Reserve (Fr, UBR). Means in the same column sharing letters are not significatively different ($p > 0.05$).

Table 3: Model selection table showing variable estimates, degrees of freedom (df), log Likelihood (logLik), AICc values, delta AICc values (ΔAICc), and weight (*w*) of each model testing the effects of landscape variables on manganese, lead and copper concentrations found in fur samples of bats from southern region of the state of Bahia, Brazil.

Table 4: Coefficients of the averaged models (for manganese and lead) and for best model (for copper) showing estimates, lower and upper limits of confidence interval (CI LL and CI UL), test statistic (*z* value), their associated *p* value, and the relative importance of each explanatory landscape variable affecting concentration of manganese, lead and copper found in fur samples of bats from southern region of the state of Bahia, Brazil. Bold letters indicate significant landscape variable for the model.

Figure 1. Location map of Bahia state, Brazil, depicting the shaded cocoa plantation study sites in Ilhéus, (black circles), Una (black triangles), Belmonte (Black squares) and the location of the sampled control sites in the Una Biological Reserve (white triangles). On the maps below, colors indicate land uses.

Figure 2: Hair metal concentration of bats from shaded cacao agroforestry sites in the different landscape context of Belmonte (Bm, Pastures and Monocultures, PM), Ilhéus (Ios, Large Farms of Shaded Cacao, LFSC), Una (Small Farms of Shaded Cacao, SFSC) and forest from Una Biological Reserve (Fr, UBR). For each plot, boxes with same letters are not significatively different ($p > 0.05$)

Figure 3: Effects of forest and open areas coverage on fur lead concentration and effect of shaded cacao agroforestry coverage upon copper fur concentration on bats from southern region of the state of Bahia, Brazil.