UNIVERSIDADE ESTADUAL DE SANTA CRUZ

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E CONSERVAÇÃO DA BIODIVERSIDADE

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BIOACUMULAÇÃO DE METAIS PESADOS EM PEQUENOS MAMÍFEROS EM AGROFLORESTAS DE CACAU SOMBREADO E REMANESCENTES FLORESTAIS NO SUL DA BAHIA, BRASIL

Ilhéus, Bahia

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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

Orientador: Dr. Ricardo Siqueira Bovendorp

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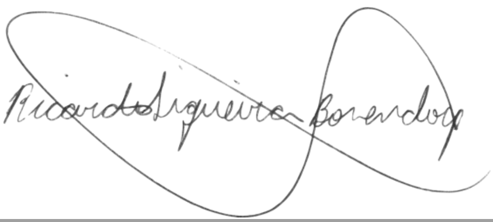

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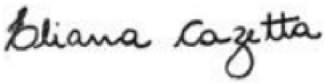

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SUMÁRIO

RESUMO

As atividades antrópicas vêm ocasionando uma série de efeitos negativos ao ambiente. Grandes extensões de florestas tropicais vêm sendo modificadas em paisagens antrópicas, que resulta em remanescentes florestais reduzidos e/ou separados por matrizes inóspitas. A Mata Atlântica é considerada um dos mais importantes biomas da região Neotropical, abrigando elevada riqueza e endemismo de espécies; e um dos mais ameaçado do mundo, em decorrência da crescente atividade agrícola. A atual crescente demanda por recursos, produção de alimentos e culturas agrícolas leva a uma redução drástica da biodiversidade e dos serviços ecossistêmicos. Uma das opções para mitigar os efeitos decorrentes das mudanças no uso da terra é a adoção de sistemas amigáveis à vida selvagem, como os sistemas agroflorestais de cacau, que integram a produção com a conservação da biodiversidade. As agroflorestas de cacau, possuem alta importância ambiental e econômica, principalmente na região tropical, abrigando uma parcela significativa de espécies de animais e plantas. No entanto, manejo do cacau é realizado pelo uso frequente de agrotóxicos que possuem metais pesados em sua composição, considerados assim esses sistemas, fontes não pontuais de poluição ambiental. O pelo animal é considerado um excelente bioindicador cumulativo de contaminação. Pequenos mamíferos são conhecidos como bioindicadores de poluição, pois alertam sobre os efeitos adversos nos ecossistemas. O objetivo principal foi determinar as concentrações de metais pesados (Pb, Ni, Cr, Cd, Mn e Cu) em pequenos mamíferos em dois diferentes tipos de habitat (agroflorestas de cacau sombreado e remanescentes florestais) na região sul do estado da Bahia, Brasil Foram capturados 34 pequenos mamíferos nas agroflorestas tradicionais de cacau e 18 nos fragmentos de Mata Atlântica. Foram estimadas, em amostras de pelo, as concentrações de chumbo (Pb), níquel (Ni), cromo (Cr), cádmio (Cd), manganês (Mn) e cobre (Cu). Descobrimos que ambos os habitats estudados estão contaminados por chumbo, uma vez que este metal pesado está acima do limite permitido para solos. As concentrações de metais pesados observadas em pelos de pequenos mamíferos de agroflorestas tradicionais de cacau foram maiores do que as observadas nos pelos de pequenos mamíferos de fragmentos de Mata Atlântica. Os marsupiais apresentaram maiores concentrações de chumbo em comparação aos roedores e isso pode estar associado aos hábitos alimentares e ao uso do habitat. A condição corporal não é afetada pelo habitat nem influencia a bioacumulação de metais pesados em pequenos mamíferos. Este é o primeiro estudo a avaliar a contaminação por metais pesados em pequenos mamíferos em agroflorestas tradicionais de cacau e fragmentos de Mata Atlântica, fornecendo assim dados importantes que indicam a presença de metais pesados nas espécies de pequenos mamíferos estudadas e, consequentemente, no ambiente onde são encontrados.

Palavras-chave: Marsupiais; Pelo; Poluição por metais pesados; Roedores; Sistema agroflorestal.

ABSTRACT

Anthropogenic activities have caused a series of negative effects on the environment. Large tracts of tropical forests have been modified into anthropic landscapes, resulting in reduced forest remnants and/or separated by inhospitable matrices. The Brazilian Atlantic Forest host a high species richness and endemism, but it is one of the most threatened ecosystems in the world, due to the growing agricultural activity. The current growing demand for resources, food production and agricultural crops leads to a drastic reduction in biodiversity and ecosystem services. One of the options to mitigate the effects resulting from changes in land use is the adoption of wildlife-friendly systems, such as cacao agroforestry systems, which integrate production with biodiversity conservation. Cacao agroforests have high environmental and economic importance, especially in the tropical region, housing a significant portion of animal and plant species. Cacao management is carried out by the frequent use of pesticides that have heavy metals in their composition, thus considering these systems, non-point sources of environmental pollution. Animal hair is considered an excellent cumulative bioindicator of contamination. Small mammals are known as bioindicators of pollution, as they provide a warning of adverse effects on ecosystems. The main objective was to determine the concentrations of heavy metals (Pb, Ni, Cr, Cd, Mn and Cu) in small mammals in two different types of habitats (traditional cacao agroforestry and Atlantic Forest fragments) in the southern region of the State of Bahia, Brazil. 34 small mammals were captured in traditional cacao agroforests and 18 in Atlantic Forest fragments. In hair samples, the concentrations of lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), manganese (Mn) and copper (Cu) were estimated. We found that in both habitats studied are contaminated by lead, since this heavy metal is above the limit allowed for soils. The concentrations of the heavy metals observed in hair of small mammals from traditional cacao agroforests were higher than the concentrations of heavy metals observed in hair of small mammals from Atlantic Forest fragments. Marsupials had higher lead concentrations compared to rodents and this could be associated with eating habits and habitat use. Body condition is not affected by habitat, nor does it influence the bioaccumulation of heavy metals in small mammals. This is the first study to evaluate heavy metal contamination in small mammals in traditional cacao agroforests and Atlantic Forest fragments, thus providing important data that indicate the presence of heavy metals of the small mammal species studied, and consequently in the environment where they are found.

Keywords: Agroforestry system; Hair; Heavy metal pollution; Marsupials; Rodents.

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Figure 3. Concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests (orange) and Atlantic Forest fragments (green) in southern Bahia, Brazil. * = Indicates a significant difference in lead (Pb) concentration between order of small mammals (Pb: p = **0.038**; Mn: p = 0.296; Cu: p = 0.094, Kruskal-Wallis test). a = Indicates a significant difference in lead (Pb) concentration between habitats (Pb: p = **0.059**; Mn: p = 0.647; Cu: p = 0.136, ANOVA rank test).

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INTRODUÇÃO GERAL

As atividades antrópicas juntamente com o crescente aumento populacional e associadas à modificação do uso da terra (BRANCALION et al., 2013; GIBSON et al., 2011), vem ocasionando uma série de efeitos negativos ao ambiente, que degradam os habitats, modificam a estrutura das comunidades biológicas e alteram as características físico-químicas do ambiente (TABARELLI et al., 2005, 2006), e consequentemente reduzem a biodiversidade (DIRZO et al., 2014). As florestas tropicais abrigam uma elevada diversidade de espécies (GIBSON et al., 2011) e atualmente, grande parte desses ecossistemas já sofreram algum tipo de alteração e/ou foram perdidos (BRANDON, 2014). Grandes extensões de florestas tropicais vêm sendo modificadas em paisagens antrópicas, que resulta em remanescentes florestais reduzidos e/ou separados por matrizes inóspitas (FAHRIG, 2013; GIBBS et al., 2010; MATSON et al., 1997; PARDINI et al., 2010; RIBEIRO et al., 2009).

Na região tropical do Brasil, a floresta Atlântica é considerada dentre os biomas brasileiros, o mais importante, abrigando elevada riqueza e endemismo de espécies; e um dos mais ameaçado do mundo, em decorrência da crescente atividade agrícola, o qual acarreta inúmeros impactos na paisagem, como fragmentação e isolamento de remanescentes florestais (FUNDAÇÃO SOS MATA ATLÂNTICA/INPE, 2017), perda da biodiversidade (FAHRIG, 2003), habitat e riqueza de espécies (VIEIRA et al., 2009). Atualmente restam apenas 11,73% da cobertura original de Mata Atlântica no Brasil (RIBEIRO et al., 2009), a qual encontra-se suprimida pela urbanização e áreas destinadas à agropecuária (BARLOW et al., 2018; CONSERVATION INTERNATIONAL, 2020; MYERS et al., 2000; VIEIRA; GARDNER, 2012).

A atual crescente demanda por recursos, produção de alimentos e cultivos agrícola leva a drástica redução da biodiversidade e dos serviços ecossistêmicos (FOLEY et al., 2005; LAURANCE; SAYER; CASSMAN, 2014), sendo uma das opções para atenuar os efeitos decorrentes das modificações do uso da terra, a adoção de sistemas amigáveis de biodiversidade (wildlife-friendly systems), como os sistemas agroflorestais, os quais integraram a produção com a conservação da biodiversidade (ABDO; VALERI; MARTINS, 2008; ATANGANA et al., 2013; FISCHER et al., 2014; PYWELL et al., 2012). Nesses sistemas, os cultivos agrícolas são manejados associados às espécies arbóreas nativas (GREENBERG; PERFECTO; PHILPOTT, 2008; MAY et al., 2008), resultando em uma atividade econômica mais sustentável, em comparação com monoculturas (SCHROTH et al., 2000), e mais amigável a biodiversidade (FARIA; SOARES-SANTOS; SAMPAIO, 2006; FARIA et al., 2006; FERREIRA et al., 2018; van BAEL et al., 2007), devido a sua complexidade estrutural, comunidades similares às das florestas do entorno, heterogeneidade de habitat (BHAGWAT et al., 2008) e manutenção da riqueza de espécies (FARIA; SOARES-SANTOS; SAMPAIO, 2006; FARIA et al., 2006, 2007; FERREIRA et al., 2018; PERFECTO et al., 2003; PINEDA et al., 2005; SAMBUICHI, 2006).

Sistemas agroflorestais, como as agroflorestas de cacau, possuem alta importância ambiental e econômica, principalmente na região tropical. No Brasil, as agroflorestas de cacau foram estabelecidas na região sul do estado da Bahia, uma das principais regiões cacaueiras (ALGER; CALDAS, 1994; DIETZ; SOUSA; BILLERBECK, 1996), a qual abrange grande parte dos remanescentes florestais de Mata Atlântica restante no nordeste brasileiro. Estes remanescentes florestais são considerados centros de endemismo e diversificação de inúmeras espécies de fauna e flora (THOMAS et al., 1998) e encontram-se imersos em uma área composta por matriz de plantações de cacau sombreadas, popularmente conhecidas como cabrucas (JONHS, 1999).

A implantação da atividade cacaueira no sul da Bahia iniciou-se em meados do século XVIII e XIX, porém intensificou-se a partir da década de 80 (ROCHA, 2008), com a instalação das agroflorestas de cacau sombreado - cabrucas, como forma alternativa mais barata em relação ao sistema de plantio tradicional - cacau intensivo à pleno-sol. No sistema de cabrucas, há supressão do sub-bosque da floresta nativa para estabelecer o cultivo do cacau sob o sombreamento do dossel (FARIA; BAUMGARTEN, 2007; RICE; GREENBERG, 2000; SAMBUICHI, 2006; SCHROTH et al., 2011). Esta prática ocupa grandes áreas e estima-se que 700.000 hectares de plantações de cacau são cultivados na região sul da Bahia, e destes, 70% consistem no sistema de cabrucas (ARAÚJO et al., 1998). Devido a retenção de parte da estrutura, das condições ambientais e dos recursos da floresta nativa (MORI et al., 1983), este tipo de sistema agroflorestal comporta uma parcela significativa de espécies de animais e plantas (CASSANO et al., 2009; DELABIE et al., 2007; FARIA; SOARES-SANTOS; SAMPAIO, 2006; FARIA et al., 2006, 2007; HARVEY; VILLALOBOS, 2007; PARDINI, 2004; van BAEL et al., 2007), porém são sistemas mais simples (RICE; GREENBERG, 2000), com comunidades empobrecidas e predominância de espécies generalistas (DELABIE et al., 2011; FARIA et al., 2007). Apesar disso, as cabrucas são áreas importantes para atenuar os impactos da fragmentação e perda de habitat, tornando-se muitas vezes habitat secundário para parte das espécies florestais, uma vez que agroflorestas de cacau são menos prejudiciais para a biodiversidade quando comparado a outros tipos de cultivo (SCHROTH et al., 2011).

O cacau (*Theobroma cacao* L.) é uma planta arbórea perene, muito suscetível a pragas e doenças fúngicas, como a vassoura de bruxa (*Moniliophthora perniciosa*), a podridão-parda (*Phytophthora spp.)* e o mal do facão (*Ceratocystis cacaofunesta*). O manejo do cacau nas agroflorestas para controle dessas doenças usualmente realizado pelo uso frequente de pesticidas, aplicados sob as folhas do cacau, os quais apresentam elevados teores de metais pesados na sua composição (GIMENO-GARCÍA; ANDREU; BOLUDA,1996; JU et al., 2007; LÓPEZ-CARNELO; DE MIGUEZ; MARBÁN, 1997). Os fungicidas comumente utilizados são os fungicidas à base de cobre, entre eles o óxido de cobre (DEBERDT et al., 2008; TONDJE et al., 2007), hidróxido de cobre e cúprico de contato, os quais são os pesticidas mais largamente utilizados para controle de pragas e doenças em sistemas de produção de cacau (ADABE; NGO-SAMNICK 2014; OLUJIDE; ADEOGUN 2006); além dos fertilizantes à base de fosfato, como o fosfanato de potássio sistêmico. As aplicações contínuas desses agroquímicos no cacau tem sido associada com o acúmulo de inúmeros metais pesados no solo (AIKPOKPODION; LAJIDE; AIYESANMI, 2010; FAN et al., 2011; HIPPLER et al., 2018; INTERNATIONAL ORGANIZATION FOR COCOA - IOC, 2012; KOMÁREK et al., 2009; WORLD COCOA FOUNDATION - WCF, 2012), resultando em risco a saúde ambiental e humana (ALLOWAY, 1995).

Atualmente, há poucas informações sobre a extensão da poluição ambiental como consequência de agroquímicos à base de metais pesados usados no cacau. Estudos demonstram que o uso não regulamentado desses compostos é danoso ao meio ambiente, animais selvagens, plantações e ao ser humano, já que gera poluentes orgânicos persistentes (ASOGWA; DONGO, 2009). Anualmente são aplicados cerca de 2,3 milhões de kg de 1600 pesticidas (PIMENTEL, 1995), sendo que apenas 15% dos pesticidas atingem seus organismos-alvos, enquanto os 85% restantes acabam contaminando o ambiente (AIKPOKPODION; LAJIDE; AIYESANMI, 2010; AIKPOKPODION et al., 2013; MARGNI et al., 2002; SIMON-SYLVESTRE; FOURNIER, 1979; WARDLE; PARKINSON, 1990). Assim, sistemas agroflorestais de cacau são considerados fontes não pontuais de poluição ambiental, principalmente por metais pesados (ALLOWAY; AYRES, 1998), porém a composição química do cacau irá variar também decorrente da região do cultivo (ARÉVALO-GARDINI et al., 2017; BERTOLDI et al., 2016) e de características químicas do solo (DE ARAUJO et al., 2017). A presença de altos teores de metais pesados nos grãos de cacau pode prejudicar a exportação dos mesmos, uma vez que reduzem sua qualidade (OVACO VACA; PINEDA LLANES, 2011; ROMERO-ESTÉVEZ et al., 2019), representando assim uma ameaça aos produtores de cacau, além de serem nocivos à saúde humana.

Metais pesados são compostos químicos com peso específico superior a 5 g/cm^3, altamente reativos, acumulativos, não degradáveis e persistentes com capacidade de contaminação e potencial de toxicidade ao meio ambiente (ALLOWAY, 2013; DOMINGO, 1994; DUFFUS, 2002; FOWLER, 1993), mesmo em concentrações muito baixas, sendo de origem natural (PEREIRA et al., 2006) e/ou provenientes de ações antrópicas (COVARRUBRAS; PEÑA CABRIALES, 2017; PHILLIPS, 1977), como descargas de efluentes, mineração, precipitações (NOVOTNY, 1995), atividades industriais (SCHRECK et al., 2012), combustão de combustíveis fósseis, tráfego (LI et al., 2004; VIDAL et al., 2004) e insumos agrícolas – fertilização, aplicação de pesticidas (CHAVEZ et al., 2015; GRAMLICH et al., 2017; MENDES et al., 2006; DE OLIVEIRA et al., 2009; ZARCINAS et al., 2004).

Os metais pesados são classificados em essenciais e não essenciais. Os metais pesados essenciais participam de processos metabólicos que regulam a produção de energia e o bom funcionamento do organismo (LIUZZI; COUSINS, 2004; RASHED; SOLTAM, 2005; WANG et al., 2011), podendo ser tóxicos em elevadas concentrações ou quando desprovidos no organismo (DAMEK-POPRAWA; SAWICKA-KAPUSTA, 2003; HOMADY et al., 2002; NORDBERG et al., 2007). Entre os metais pesados essenciais, podemos citar o níquel (Ni), cromo (Cr), manganês (Mn) e cobre (Cu). Já os metais pesados não essenciais são aqueles não requeridos pelos organismos, uma vez que não apresentam função metabólica conhecida (GAUTHIER et al., 2014; RAMM, 2015). Dentre os metais não essenciais, podemos citar o cádmio (Cd) e chumbo (Pb), os quais são considerados uns dos elementos mais perigosos do mundo (CASTEBLANCO, 2018; ENGBERSEN et al., 2019), uma vez que são extremamente tóxicos mesmo em concentrações baixas (DAMEK-POPRAWA; SAWICKA-KAPUSTA, 2004; DROUHOT et al., 2014).

A contaminação, mesmo em pequenas proporções, por metais pesados é inevitável em plantas, animais e humanos, sendo incorporados por meio da ingestão de alimentos e água contaminados, inalação e/ou absorção cutânea (LUCHO-CONSTANTINO et al., 2005). Esses compostos causam impactos na saúde dos organismos e do ambiente, devido a sua alta mobilidade, persistência e poder acumulativo (ENGBERSEN et al., 2019; REYS et al., 2016), gerando sérios riscos à saúde humana e dos animais selvagens, além da preocupação com a segurança alimentar (ALBARRCÍN; CONTRETAS; HENAO, 2019; BALIGAR; FAGERIA; ELRASHIDI, 1998; BENAVIDES; GALLEGO; TOMARO, 2005; CHAVEZ et al., 2015; FERRANTE et al., 2017a, b; MARSCHNER, 2012). Altas concentrações de metais pesados, quando presentes, no corpo dos organismos, podendo resultar em efeitos comportamentais, neurológicos, carcinogênicos, mutagênicos, cardíacos, morte por envenenamento, além de complicações em órgãos vitais, como rins, fígado, cérebro, ossos, pulmões, pâncreas (ARÉVALO-GARDINI et al., 2017).

Os seres vivos são incapazes de excretar metais pesados de maneira eficiente, ocasionando na bioacumulação destes nos tecidos e a disseminação em larga escala nos ecossistemas, acarretando na biomagnificação de um nível trófico para outro ao longo da teia alimentar (BAYKOV; STOYANOV;

GUGOV, 1996; DEMIREZEN; URUÇ, 2006; FRINK, 1996; LOSKA; WIECHUŁA, 2003; MALHAT et al., 2012; MARCHESELLI; SALA; MAURI, 2010; MERIAN, 1991), ocasionado graves danos ecológicos e de saúde ao ecossistema (MALIK, 2004). Uma vez presentes no corpo dos organismos, os metais pesados são armazenados nos tecidos moles, penas e pelos dos animais (GOEDE; DE BRUIN, 1984, 1986; NAVARRO-AVIÑÓ; AGUILAR ALONSO; LÓPEZ-MOYA, 2007; PHELPS et al., 1980).

O pelo animal é considerado um excelente bioindicador cumulativo de contaminação, sendo um método não invasivo apropriado para o monitoramento de metais pesados (COVACI et al., 2002; JASPERS et al., 2010; LIANG et al., 2014). Nos últimos anos, o seu uso para o biomonitoramento da exposição a contaminantes tem aumentado, devido a este ser um método alternativo não destrutivo (DAUWE et al., 2002; D'HAVÉ et al., 2006; TÊTE et al., 2014), que ocasiona em estresse mínimo aos indivíduos amostrados. Além disso, o pelo permite a análise dos níveis de contaminação em espécies ou populações ameaçadas, monitoramento sucessivo dos níveis de contaminantes de populações e indivíduos durante um longo período (D'HAVÉ et al., 2006), além de ser considerado eticamente aceito, econômica e facilmente aplicável (ALI et al., 2013; JASPERS et al., 2019; POMA; MALARVANNAN; COVACI, 2020; SCHRAMM, 2008; SONNE et al., 2020; SUN et al., 2019).

O pelo reflete a acumulação e concentração de metais pesados dos meses e até anos anteriores presentes no organismo dos animais (RAY et al., 1997), já que a queratina, constituinte principal do pelo, encontra-se continuamente em contato com a corrente sanguínea durante seu o crescimento, incorporando assim os metais presentes (BEERNAERT et al., 2007; BURGER; MARQUEZ; GOCHFELD, 1994; McLEAN et al., 2009; VERMEULEN et al., 2009; TÊTE et al., 2014; WIIG; RENZONI; GJERTZ, 1999). Além disso, eles podem ser utilizados como indicadores das concentrações de metais pesados em outros tecidos (AL-SHAHRISTANI; SHIHAB; AL-HADDAD, 1976; BURGER; MARQUEZ; GOCHFELD, 1994; FRANCIS et al., 1982), como fígado e rim. Em mamíferos, estudos afirmam que as concentrações de metais pesados refletidos nos pelos estão correlacionadas aos níveis presentes nos tecidos moles e também no ambiente (McLEAN et al., 2009; RASHED; SOLTAN, 2005).

Animais selvagens estão naturalmente expostos a metais pesados (KÅLÅS; STEINNES; LIERHAGEN, 2000), os quais, dependendo da sua disponibilidade no ambiente, podem ser provenientes da absorção dérmica, alimentação ou inalação. Eles vêm sendo cada vez mais utilizados como sentinelas ambientais para monitorar a biodisponibilidade de contaminantes, uma vez que ajudam a compreender os efeitos no ambiente decorrente da poluição por metais pesados (ALLEVA et al., 2006; BEERNAERT et al., 2007; CARSON, 1962; NEWMAN, 1998; O'BRIEN; KANEENE; POPPENGA, 1993; PEAKALL, 1992; STAHL Jr., 1997; TALMAGE; WALTON, 1991; van der SCHALIE et al., 1999), fornecendo informações sobre a viabilidade e equilíbrio ambiental. O biomonitoramento dos níveis de metais pesados além de fornecer informações sobre estado de saúde dos ecossistemas, é útil ainda para avaliações de risco à saúde humana.

Os mamíferos, no geral, são conhecidos como indicadores convenientes de poluição (BRAIT; ANTONIOSI FILHO; FURTADO, 2009), pois fornecem um alerta de efeitos adversos nos ecossistemas. Estes, por possuírem uma vida relativamente longa, sofrem com os efeitos ambientais durante um longo período, acumulando concentrações de metais nos seus tecidos (MARQUES et al., 2008; PEREIRA et al., 2006; SÁNCHEZ-CHARDI; GARCÍA-PANDO; LÓPEZ-FUSTER, 2013; SÁNCHEZ-CHARDI et al., 2007a), proveniente principalmente da sua dieta. Entre as espécies sentinelas para biomonitoramento ambiental, os pequenos mamíferos terrestres são o principal grupo de mamíferos utilizado, já que estes respondem a uma gama de mudanças nos ecossistemas (SÁNCHEZ-CHARDI et al., 2007 a, b; SÁNCHEZ-CHARDI; NADAL, 2007; SHEFFIELD et al., 2001; TALMAGE; WALTON, 1991).

Os pequenos mamíferos consistem no grupo de mamíferos de peso inferior a 1 quilo, compostos pelas ordens Chiroptera (morcegos), Didelphimorphia (marsupiais) e Rodentia (roedores). Esses animais habitam quase todos os habitats terrestres e representam cerca de 80% de toda a riqueza de mamíferos do Brasil (PAGLIA et al., 2012). Aproximadamente 286 espécies de pequenos mamíferos já foram descritas no Brasil (PAGLIA et al., 2012), sendo 124 delas para a Mata Atlântica – 94 espécies de roedores e 30 espécies de marsupiais (BOVENDORP; McCLEERY; GALETTI, 2017). Os pequenos mamíferos cumprem importantes papéis funcionais e tróficos nos ecossistemas terrestres, entre eles: ocupam uma variedade de nichos; desempenham um papel importante na ciclagem de nutrientes; podem influenciar presas de invertebrados e comunidades de plantas; e são presas para uma gama de predadores vertebrados e alimento para inúmeros invertebrados (LEVENGOOD; HESKE, 2008).

Os roedores e marsupiais têm sido usados com frequência como bioindicadores de poluição (BEERNAERT et al., 2007; D'HAVÉ et al., 2006; MA, 1989; MA; DENNEMAN; FABER, 1991; MARCHESELLI; SALA; MAURI, 2010; McLEAN et al., 2009; PEREIRA et al., 2006; TALMAGE; WALTON, 1991), pois por meio da análise de seus tecidos é possível estimar e avaliar a concentração de contaminantes ao longo da cadeia trófica, o grau de perturbação antrópica em habitats naturais e os riscos que estes trazem à saúde humana. O acúmulo de metais pesados em pequenos mamíferos ocorre principalmente através da dieta e ingestão de alimento ou água contaminados (HUNTER; JOHNSON; THOMPSON, 1987 a, b, 1989; MA; DENNEMAN; FABER, 1991; MA, 1994; SHEFFIELD et al., 2001).

Os pequenos mamíferos constituem um grupo modelo para o monitoramento ambiental, pois possuem tamanho corporal pequeno, alta taxa metabólica (LEVENGOOD; HESKE, 2008; MARQUES et al., 2007; REINECKE et al., 2000; SÁNCHEZ-CHARDI; LÓPEZ-FUSTER; NADAL, 2007; SÁNCHEZ-CHARDI et al., 2007 a, b; SÁNCHEZ-CHARDI; NADAL, 2007; SÁNCHEZ-CHARDI; RIBERIO; NADAL, 2009; SÁNCHEZ-CHARDI; LÓPEZ-FUSTER, 2009), ampla distribuição geográfica e alta abundância, baixa taxa de migração (ZARRINTAB; MIRZALI, 2017), pequena área de vida (1 a 2 hectares), expectativa de vida adequada (vários meses até 2 anos de vida) e hábitos alimentares generalizados, a fim de assegurar a detecção precisa dos efeitos da exposição a poluentes em uma janela temporal local (KOMARNICKI, 2000; MARCHESELLI; SALA; MAURI, 2010). Além disso, esses animais são facilmente coletados e identificados, sendo assim, considerados candidatos ideais e confiáveis para monitorar a extensão de exposição à poluição ambiental (LEVENGOOD; HESKE, 2008; SÁNCHEZ-CHARDI et al., 2007 a, b; SÁNCHEZ-CHARDI; NADAL, 2007).

Ecossistemas terrestres que possuem populações de pequenos mamíferos apresentam-se geralmente contaminados por metais pesados potencialmente tóxicos provenientes de insumos agrícolas, como pesticidas e/ou fertilizantes, sendo geralmente absorvidos pelas plantas, e mais tarde, encontrados em tecidos de animais e humanos. Os roedores e marsupiais são usados como substitutos ou proxy de mamíferos para humanos (DAMEK-POPRAWA; SAWICKA-KAPUSTA, 2003; SHORE; RATTNER, 2001), uma vez que por meio da análise de seus tecidos, podemos obter informações sobre a contaminação ambiental e avaliação de risco de exposição para os humanos que habitam locais contaminados (DAMEK-POPRAWA; SAWICKA-KAPUSTA, 2003; O'BRIEN; KANEENE; POPPENGA, 1993), já que esses animais são geneticamente semelhantes aos humanos (BEERNAERT et al., 2007; FESTA et al., 2003; LOURENÇO et al., 2013; MARCHESELLI; SALA; MAURI, 2010).

Apesar dos pequenos mamíferos cumprirem importantes papéis funcionais e tróficos nos ecossistemas terrestres, existem ainda poucos dados sobre os efeitos da poluição ambiental nesse grupo. Assim, conhecer o impacto de diferentes atividades humanas nas populações naturais, e em particular, nos pequenos mamíferos, é de extrema importância para estudos de conservação e gestão local, pois estes ajudam a obter informações sobre a tolerância do ecossistema a certos tipos de poluentes, a eficiência dos mecanismos naturais de autorregulação, além de avaliar os riscos à saúde humana e ambiental (NATIONAL RESEARCH COUNCIL - NRC, 1991; PEREIRA et al., 2006).

OBJETIVOS

OBJETIVO GERAL

O presente trabalho teve como objetivo determinar as concentrações de metais pesados em pequenos mamíferos (marsupiais e roedores) em dois diferentes tipos de habitat (agroflorestas de cacau sombreado e remanescentes florestais) na região sul do estado da Bahia, Brasil.

OBJETIVOS ESPECÍFICOS

- Analisar as concentrações de metais pesados entre as agroflorestas de cacau sombreado e remanescentes florestais, usando o pelo de pequenos mamíferos como proxy para identificar as diferenças entre os habitats.
- Analisar as concentrações de metais pesados entre as ordens de pequenos mamíferos (Didelphimorphia e Rodentia).
- Analisar a condição corporal dos pequenos mamíferos entre as agroflorestas de cacau sombreado e remanescentes florestais.
- Analisar se as concentrações de metais pesados influenciam a condição corporal dos pequenos mamíferos.

HIPOTESES

Diante esses objetivos, testamos as seguintes hipóteses:

(1) Haverá bioacumulação de metais pesados em pequenos mamíferos de áreas de agroflorestas de cacau sombreado, devido ao uso frequente e não regulamentado de agroquímicos empregados nesse tipo de cultivo, já que o manejo é realizado por meio de aplicações de pesticidas, inseticidas e fertilizantes para controle de doenças e pragas, bem como para auxiliar no aumento da produtividade do cacau (AIKPOPODION et al., 2013; THOMAS, 2015), os quais concentram altos teores de metais pesados na sua composição (GIMENO-GARCÍA; ANDREU; BOLUDA,1996; JU et al., 2007; LÓPEZ-CARNELO; DE MIGUEZ; MARBÁN, 1997);

(2) Roedores irão apresentar concentrações de metais pesados mais elevadas em relação aos marsupiais, já que este grupo de pequenos mamíferos geralmente possuem hábito terrestre, tendo assim contato direto e constante com o solo, o qual recebe grande parte dos agroquímicos aplicados as culturas (AIKPOKPODION; LAJIDE; AIYESANMI, 2010; AIKPOKPODION et al., 2013; MARGNI et al., 2002; SIMON-SYLVESTRE; FOURNIER, 1979; WARDLE; PARKINSON, 1990);

(3) Pequenos mamíferos capturados nas áreas de remanescentes florestais de Mata Atlântica apresentarão condições corporais maiores, devido ao fato desse habitat ser livre de aplicações de agroquímicos, uma vez que esta área não tem práticas agrícolas e devido a disponibilidade hídrica que favorece a qualidade e quantidade de recursos alimentares, e como consequência a condição corporal (D’ANDREA et al., 2007; HARTMANN; HARTMANN; MARTINS, 2009);

(4) A bioacumulação de metais pesados em pequenos mamíferos influenciará a condição corporal dos mesmos, uma vez que concentrações de metais pesados nos organismos destes animais resultam em distúrbios metabólicos e/ou comportamentais (AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY – ATSDR, 2007; CARRINGTON; BOLGER, 1992; EUROPEAN FOOD SAFETY AUTHORITY – EFSA, 2012; WORLD HEALTH ORGANIZATION – WHO, 2011).

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CHAPTER 1

Costa, L.S., Souza, A. P. de, **Bovendorp, R.S.** HEAVY METALS IN HAIR OF SMALL MAMMALS FROM THE CACAO AGROFORESTRY AND BRAZILIAN ATLANTIC FOREST.

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Title: Heavy Metals in Hair of Small Mammals from the Cacao Agroforestry and Brazilian Atlantic Forest

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Highlights

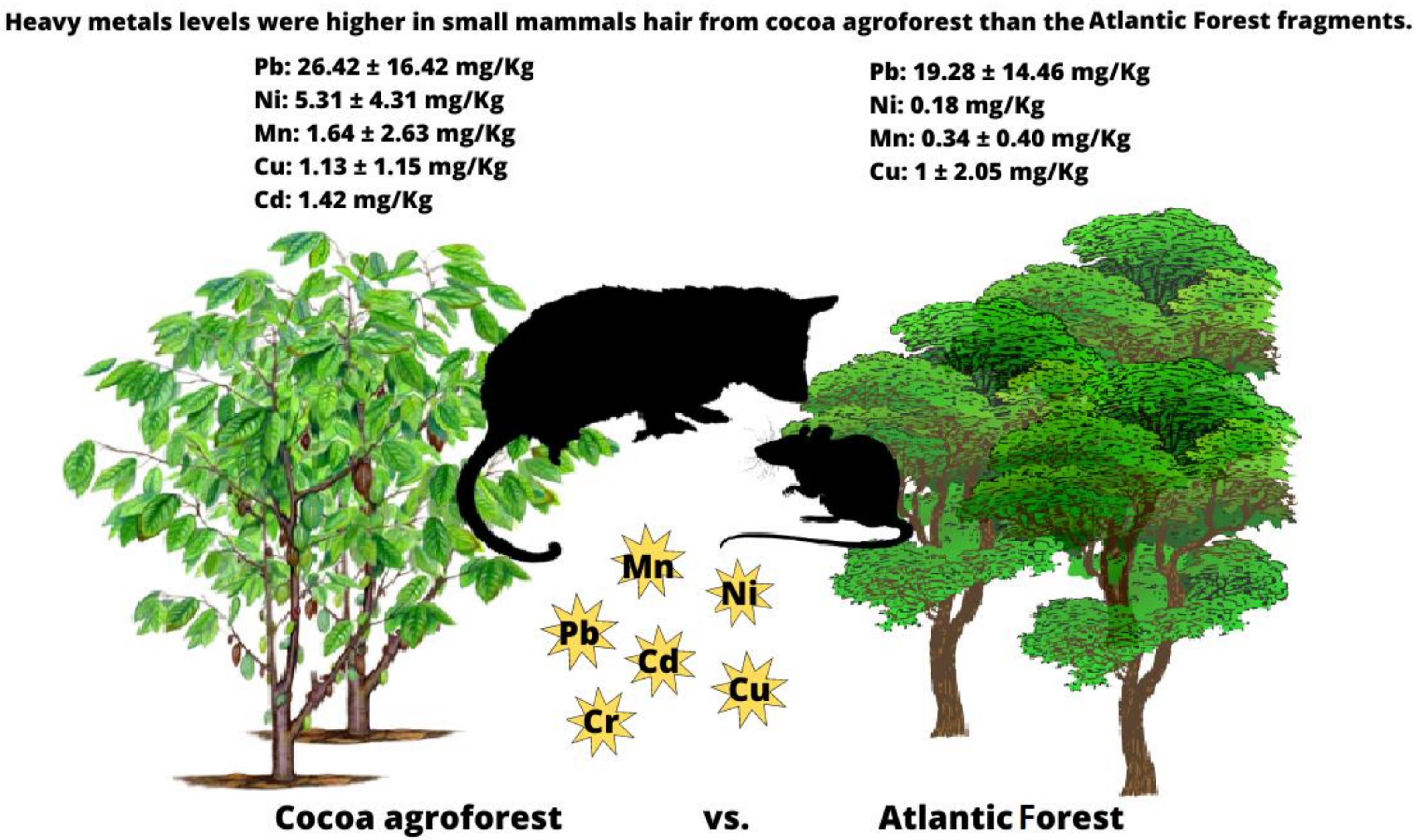
- This is the first report of heavy metals levels in Neotropical small mammals.
- Levels of Ni, Pb, Cd, Mn and Cu were measured in small mammal's hair.
- Heavy metals levels were higher in cacao agroforest than the Atlantic Forest fragments.
- Both habitats studied are contaminated by lead, since this heavy metal is above the limit allowed for soils.
- Body condition of small mammals does not influence heavy metals hair concentrations.

Abstract

The current growing demand for resources, food production and agricultural crops leads to a drastic reduction in biodiversity and ecosystem services. One of the options to mitigate the recurring effects of land use changes is the adoption of wildlife-friendly systems, such as cacao agroforestry systems, which integrated production with biodiversity conservation. The management of cacao is carried out by the frequent use of pesticides which have heavy metals in their composition, which are considered non-point sources of environmental pollution. Animal hair is considered an excellent cumulative bioindicator of contamination. Small mammals are known as bioindicators of pollution, as they provide a warning of adverse effects on ecosystems. The main objective was to determine the concentrations of Pb, Ni, Cr, Cd, Mn and Cu in small mammals in traditional cacao agroforest and Atlantic Forest fragments in the southern region of Bahia, Brazil. 34 small mammals were captured in the traditional cacao agroforests and 18 in the Atlantic Forest fragments. We found that in both habitats studied are contaminated by lead, since this heavy metal is above the limit allowed for soils. The concentrations of the heavy metals observed in hair of small mammals from traditional cacao agroforests were higher than the concentrations of heavy metals observed in hair of small mammals from Atlantic Forest fragments. Marsupials had higher lead concentrations compared to rodents and this could be associated with eating habits and habitat use. Body condition is not affected by habitat, nor does it influence the bioaccumulation of heavy metals in small mammals. This is the first study to evaluate heavy metal contamination in small mammals in traditional cacao agroforests and Atlantic Forest fragments, thus providing important data that indicate the presence of heavy metals of the small mammal species studied, and consequently in the environment where they are found.

Keywords: Agroforestry system; Cacao; Heavy metal pollution; Mammals; Marsupials; Rodents.

Graphical Abstract



Introduction

Anthropogenic activities, together with the growing population and associated with land use modification (Brancalion et al., 2013; Gibson et al., 2011), have caused a series of negative environmental effects, which degrade habitats, modify the structure of biological communities, alter the physical-chemical characteristics of the environment (Tabarelli et al., 2005, 2006), reduce the resilience of biodiversity and result in the extinction of numerous species (Dirzo et al., 2014). One of the main drives of biodiversity is agriculture, where the land is managed with pesticides and fertilizers to grow food and commodities for the population (Foley et al., 2011; Green et al., 2005; Popp et al., 2012; Sud, 2020).

The current growing demand for resources, food production and agricultural crops leads to a drastic reduction in biodiversity and ecosystem services (Foley et al., 2005; Laurance et al., 2014), and one of the options to mitigate the recurring effects of land use changes is the adoption of wildlife-friendly systems, such as agroforestry, which integrated production with biodiversity conservation (Abdo et al., 2008; Atangana et al., 2013; Fischer et al., 2014; Pywell et al., 2012). Agroforestry systems, such as cacao (*Theobroma cacao* L.) agroforestry, have high environmental and economic importance, especially in the tropical region, as they include a significant portion of animal and plant species (Cassano et al., 2009; Delabie et al., 2007; Faria et al., 2006 a, b, 2007; Harvey and Villalobos, 2007; Pardini, 2004; Van Bael et al., 2007). However, they are simpler systems (Rice and Greenberg, 2000), with impoverished communities and a predominance of generalist species (Delabie et al., 2011; Faria et al., 2007). In cacao agroforestry, management to control of fungal pests and diseases, such as "witches' broom disease" (*Moniliophthora perniciosa*), “brown rot disease” (*Phytophthora* spp*.)* and "ceratocystis wilt of cacao" (*Ceratocystis cacaofunesta*), is usually carried out by the frequent use of pesticides, applied under leaves, which have high levels of heavy metals in their composition (Gimeno-García et al., 1996; Ju et al., 2007; López-Carnelo et al., 1997). Commonly used fungicides are copper-based fungicides, including Copper oxide (Deberdt et al., 2008; Tondje et al., 2007), Copper hydroxide and contact cupric (Olujide and Adeogun, 2006; Adabe and Ngo-Saminick, 2014), in addition to phosphate-based fertilizers, such as systemic potassium phosphate. The continuous application of these agrochemicals in cacao has been associated with the accumulation of numerous heavy metals, like lead, manganese, copper and cadmium, in the soil and trees of cacao in many parts of the world (Aikpokpodion et al., 2010; Arévalo-Gardini et al., 2017; Arham et al., 2017; Fan et al., 2011; Hippler et al., 2018; International Organization for Cocoa – IOC, 2012; Komárek et al., 2009; World Cocoa

Foundation – WCF, 2012), resulting in a risk to environmental and human health (Alloway, 1995).

Currently, there is little information on the extent of environmental pollution as a result of heavy metal-based agrochemicals used in cacao. Studies show that the unregulated use of these compounds is harmful to the environment, wild animals, crops and humans, as it generates persistent organic pollutants (Asogwa and Dongo, 2009). Approximately 2.3 million kg of 1600 pesticides are applied annually (Pimentel, 1995), with only 15% of pesticides reaching their target organisms (Aikpokpodian et al., 2010; Aikpokpodion et al., 2013; Margni et al., 2002; Simon-Sylvestre and Fournier, 1979; Wardle and Parkinson, 1990). Thus, cacao agroforestry systems are considered non-point sources of environmental pollution, mainly by heavy metals (Alloway and Ayres, 1997), but the chemical composition of cacao will also vary depending on the region of cultivation (Arévalo-Gardini et al., 2017; Bertoldi et al., 2016) and soil chemical characteristics (De Araujo et al., 2017). Heavy metals are highly reactive, accumulative, non-degradable and persistent chemical compounds with potential for contamination to the environment (Alloway, 2013; Domingo, 1994; Duffus, 2002; Fowler, 1993), being of natural origin and/or from human actions (Covarrubras and Peña Cabriales, 2017; Pereira et al., 2006; Phillips, 1977). Contamination, even in small proportions, by heavy metals is inevitable, being incorporated through the ingestion of contaminated food and water, inhalation and/or cutaneous absorption (Lucho-Constantino et al., 2005), causing impacts on environment and human health, in addition to concerns about food safety (Albarrcín et al., 2019; Baligar et al., 1998; Benavides et al., 2005; Chavez et al., 2015; Ferrante et al., 2017 a, b; Marschner, 2012).

Animal hair is considered an excellent cumulative bioindicator of contamination, being an appropriate non-invasive method for monitoring heavy metals (Covaci et al., 2002; Jaspers et al., 2010; Liang et al., 2014), as it reflects exposure to these elements (Goede and De Bruin, 1986; Phelps et al., 1980). In recent years, its use for biomonitoring has increased, because it is a non-destructive alternative method (Dauwe et al., 2002; D'Havé et al., 2006; Tête et al., 2014), which causes minimal stress to the individuals sampled. The hair allows the analysis of contamination levels in endangered species or populations, successive monitoring of the levels of contaminants in populations and individuals over a long period of time (D'Havé et al., 2006; Ray et al., 1997), in addition to being considered ethically accepted, economic and easily applicable (Ali et al., 2013; Jaspers et al., 2019; Poma et al., 2020; Schramm, 2008; Sonne et al., 2020; Sun et al., 2019). Furthermore, they can be used as indicators of heavy metal concentrations in other tissues, like blood, liver and kidneys (Al-Shahristani et al., 1976; Burger et al 1994; Francis et al., 1982; McLean et al., 2009; Vermeulen et al., 2009). In mammals, studies claim that the concentrations of heavy metals reflected in the hair are correlated with the levels present in soft tissue, such as liver and kidneys, and also in the environment (McLean et al., 2009; Rashed and Soltan, 2005).

Small mammals constitute a model group for environmental monitoring (Beernaert et al., 2007; D'Havé et al., 2006; Ma, 1989; Ma et al., 1991; Marcheselli et al., 2010; McLean et al., 2009; Pereira et al., 2006; Talmage and Walton, 1991), because through the analysis of their tissues it is possible to estimate and evaluate the concentration of contaminants along the trophic chain, the degree of human disturbance in natural habitats, in addition to estimating the risks they bring to human health, since that rodents and marsupials are used as substitutes or proxy of mammals for humans (Damek-Poprawa and Sawicka-Kapusta 2003; O'Brien et al., 1993; Shore and Rattner 2001). Despite this, there are still few data on the effects of environmental pollution on this group of mammals. Knowing the impact of different human activities on natural populations, and in particular on small mammals, is extremely important for conservation studies and local management, as they help to obtain information about the ecosystem's tolerance to certain types of pollutants, the efficiency of natural self-regulation mechanisms, in addition to assessing the risks to human and environmental health (National Research Council – NRC, 1991; Pereira et al., 2006).

The main objective of this study was to determine the concentrations of heavy metals in small mammals in two different types of habitats (traditional cacao agroforest and Atlantic Forest fragments) in the southern region of the state of Bahia, Brazil. First, we analyzed the concentrations of heavy metals between traditional cacao agroforest and Atlantic Forest fragments, used the small mammal's hair as a proxy to identify differences between habitats. Second, we analyzed the concentrations of heavy metals regarding the order of small mammals (Didelphimorphia and Rodentia). Third, we analyzed the body condition of small mammals between traditional cacao agroforest and Atlantic Forest fragments. Finally, we analyzed whether the concentrations of heavy metals influence the body condition of small mammals.

Materials and methods

Study area

The study was conducted in Atlantic Forest fragments and in a Traditional cacao agroforest, known regionally as cabruca, which are cacao (*Theobroma cacao* L.) plantation under to the shade of native trees in the Atlantic Forest in southern Bahia, Brazil. The study was carried out in 12 traditional cacao agroforests and 12 Atlantic Forest fragments, covering the municipalities of Belmonte (15° 51′ 35″ S, 38° 53′ 25″ W), Ilhéus (14° 47′ 50″ S, 39° 2′ 8″ W) and Una (15º 17' 36'' S, 39º 04' 31'' W) in the State of Bahia (FIGURE 1). The southern region of the State of Bahia has a hot and humid climate, with no dry season. According to Köppen's classification, the region's climate is characterized as Af type, with average annual precipitation between 1.200 and 1.800 mm (Mori et al., 1983) and annual average temperature varies from 23º to 24º C (Alvares et al., 2013).

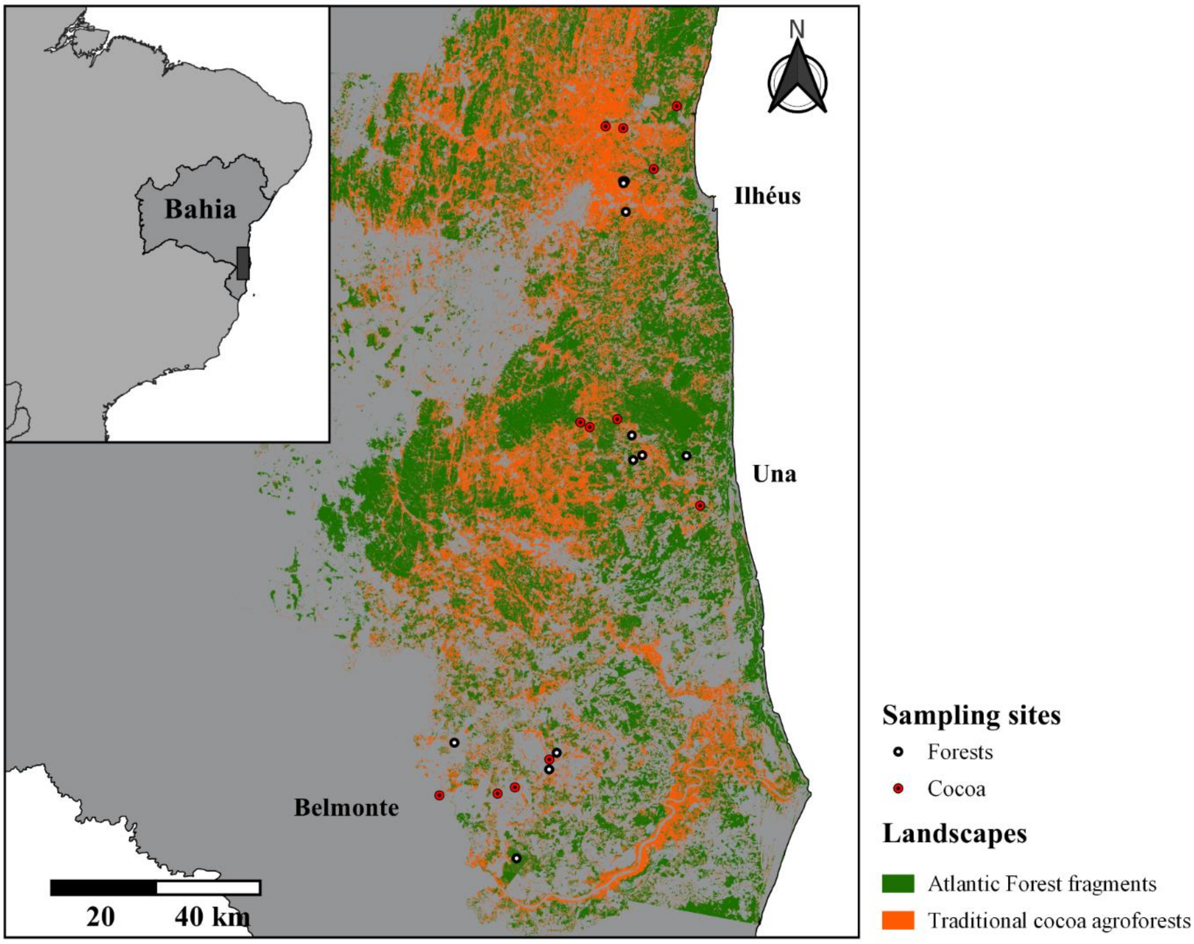


Figure 1. Distribution of sampling sites within the Atlantic Forest, including 12 traditional cacao agroforests (cabrucas) and 12 forest fragments in Ilhéus (upper panel), Una (middle panel) and Belmonte (bottom panel), in southern Bahia, Brazil.

The Atlantic Forest in southern Bahia is located between the Jequitinhonha and Contas rivers, and has a rich diversity of flora and fauna, represented by a mosaic of native forests, cultivation of cacao, eucalyptus, rubber and pastures (Pardini et al., 2009; Tabarelli et al., 2010), and harbors largest Atlantic Forest fragments in northeast Brazil, being considered one of the main centers of endemism of the Brazilian Atlantic Forest (Myers et al., 2000; Thomas et al., 1998). The municipalities of Belmonte, Ilhéus and Una are immersed in an area composed of a matrix of cacao plantations, and its protection is extremely important for biodiversity conservation (Schroth et al., 2004). The cacao production agroecosystems in the southern region of Bahia are important for soil, water and biodiversity conservation, as they contain a great floristic diversity and have proved to be important for the local fauna and can serve as a shelter or ecological corridor for the remaining species in the region, mitigating the effects of habitat loss (Faria et al., 2007; Tisovec et al., 2014). However, the landscape of these municipalities has a different landscape composition and degree of conservation of Atlantic Forest fragments (Faria et al., 2021; Ramos et al., 2022). The landscape of the municipality of Belmonte, the landscape is dominated by pastures and monocultures, such as eucalyptus, and few traditional cacao agroforests and Atlantic Forest fragments, which are highly degraded with few remnants remaining (Pessoa et al., 2017). In Ilhéus, the landscape is formed by large traditional cacao agroforests, which are managed by large producers, and in addition to few Atlantic Forest fragments. And in Una, the landscape is made up of small farms of shaded cocoa agroforests, associated with other fruits crops, generally small and managed by minor owners and local families. This region is better preserved and has largest forest remnants in compare than Belmonte and Ilhéus (Morante-Filho et al., 2016).

Sampling of small mammals

Sampling was performed using live traps (Sherman® - 25×8×9 cm and Tomahawk® - 18×18×39 cm). The sampling design were arranged in a grid composed of three parallel transects in each habitat (Traditional cacao agroforests and Atlantic Forest fragments), each about 100 meters long, separated by 20 meters, and 10 trapping points, 10 meters apart. Traps were placed on the ground and in the understory (approximately 1.5 meter from the ground) alternately in the trapping points, as much as the type, in order to maximize the capture of the arboreal species. All traps were baited with a mixture of sardines, peanuts, banana, and cornmeal. In the total, 30 traps were distributed in each sample site, with 360 traps in the traditional cacao agroforests and 360 traps in the Atlantic Forest remnants.

The sampling was carried out between the months of January and September 2021 in an expedition of 7 nights of trapping, totaling 2.520 trap-nights in the traditional cacao agroforests and 2.520 trap-nights in the Atlantic Forest fragments. The captured animals were identified, sexed, aged (dental formula for marsupials and pelage color, size and reproductive stage for rodents), weighed and their body length was measured. Body condition index (BCI) was obtained according to Peig and Green (2009, 2010) as a regression of body length and body weight. Samples with positive values were considered with the highest body condition, and negative values were considered with the lowest body condition. We collected around 0.004 to 0.649 grams of hair from the right rear thigh, by scraping at the base of the animal's hair. Hairs were conditioned in contamination-free polyethylene bags and frozen at -20ºC. The captured animals were managed under authorizations by SISBIO / ICMBio: 69688-4 and CEUA – UESC: 005/2019. No anesthetic was used to contain the animals and all capture and handling protocol followed the guidelines of the American Society of Mammals (Sikes, 2016).

Chemical analyses

The hair samples were thawed and weighed using an analytical balance, where the wet weights were measured. After weighing, about 4 to 64.9 mg of the samples were placed in digestion tubes (25×250 mm) of a digester block and conditioned in the fume hood (PVC hood) for digestion with nitric acid (HNO_3) – perchloric acid ($HClO_4$). This digestion is widely used in samples of animal origin, for the extraction of heavy metals (Blanchar et al., 1965; Chapman and Pratt, 1961; Johnson and Ulrich, 1959; Sarruge and Haag, 1974; Tabatabi and Bremner, 1970). Initially, 6.0 ml of nitric acid (HNO_3) were added to the samples to carry out the previous digestion. The samples 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 and the volume reduced to 0.5 / 1.0 ml of nitric acid (HNO_3). After that, 1.0 ml of perchloric acid ($HClO_4$) was added to the samples and the temperature of the digester block was gradually increased to reach 180°C. Funnels were placed covering the digestion tubes to avoid possible losses of perchloric acid ($HClO_4$) and sample extracts. It was kept at this temperature until white fumes of perchloric acid ($HClO_4$) were obtained, and the sample extract was colorless (approximately 2 hours).

Deionized water was placed in the tubes to stop digestion. Allowed the tubes to cool and made up the volume to 20 ml with deionized water. We quantified the bioaccumulation of six types of the heavy metals: Nickel (Ni), Lead (Pb), Cadmium (Cd), Chromium (Cr), Manganese (Mn) and Copper (Cu) analyzed by Atomic Absorption Spectrometer 240FS AA. Metal concentrations of heavy metals were expressed as below: mean ± standard deviation in mg/Kg. All chemical analyses were performed at the Plant Tissue Analysis Laboratory at the Federal University of Paraiba. The limit of detection (LOD) of the equipment were Ni, Pb, Cd, Cr Mn and Cu - <0,0001 mg/Kg. Analyses of procedural blanks were performed as additional quality control.

Statistical analyses

The Shapiro-Wilk test was used in order to test for normality of the data. ANOVA test was used for comparing difference in the average concentration of heavy metals in the small mammal's hair among the habitats (traditional cacao agroforests and Atlantic Forest fragments) and among the municipalities (Belmonte, Ilhéus and Una). The non-parametric Kruskal-Wallis and ANOVA rank tests were applied when the data distribution did not follow the rules of normal distribution. To analyze the ordering of heavy metals concentrations in the hair of all animals, a Principal Component Analysis (PCA) was performed. To analyze the correlation among heavy metals in all small mammals captured from the Traditional cacao agroforests and Atlantic Forest fragments, we used the Pearson's Correlation test. To compare the body condition (BCI) between the sampled habitats (traditional cacao agroforests and Atlantic Forest fragments), we used the Analysis of Covariance (ANCOVA). To compare all heavy metals between habitats (traditional cacao agroforests and Atlantic Forest fragments) and body condition index (BCI), a Permutational Analysis of Variance (PERMANOVA) was performed.

In our study, we did not have enough data to compare heavy metals concentrations between sexes and for the analyses, we choose one species of rodent, Atlantic Forest Oryzomys (*Hylaeamys seuanezi*) and marsupial, Linnaeus's Mouse Opossum (*Marmosa murina*) that occurred in both habitats (traditional cacao agroforests and Atlantic Forest fragments) and had a more than five individuals in each habitat (>5 individuals in traditional cacao agroforests and >5 individuals in Atlantic Forest fragments). Undetected values of concentration of heavy metals were excluded from the analysis. Only the concentrations of Lead (Pb), Manganese (Mn) and Copper (Cu) were used in the analysis, as these metals were the most detected in the samples (sample size >10). Significant differences were accepted at $p \leq 0.05$. All statistical analyzes were performed in the statistical environment R (R Core Team, 2022).

Results

In total, we captured 52 individuals of small mammals in Traditional cacao agroforests and Atlantic Forest fragments. Were captured in the traditional cacao agroforests 34 small mammals, being 18 marsupials (4 species: *Didelphis aurita, Marmosa murina, Marmosops incanus* and *Metachirus nudicaudatus*) and 16 rodents (8 species: *Akodon cursor, Guerlinguetus ingrami, Hylaeamys seuanezi, Oecomys catherinae, Rattus* sp., *Rhipidomys mastacalis*, Spp and *Thaptomys nigrita*). In the Atlantic Forest fragments were captured 18 small mammals, being 10 marsupials (3 species: *Didelphis aurita, Marmosa murina* and *Marmosops incanus*) and 8 rodents (3 species: *Akodon cursor, Hylaeamys seuanezi* and *Phyllomys pattoni*) (Supplementary Material – TABLE 1). Marsupials were more abundant in both habitats. The dominant marsupial species was the Linnaeus's Mouse Opossum *Marmosa murina*. On the other hand, the dominant rodent species was the Atlantic Forest Oryzomys *Hylaeamys seuanezi*.

The body weight and body length in marsupials (Didelphimorphia) and rodents (Rodentia) showed similar among themselves and in relation to habitats (TABLE 1). The weight and length of marsupials and rodents from Atlantic Forest fragments were greater than those from traditional cacao agroforests (TABLE 1). The body condition index (BCI) of the marsupials was positive values in both habitats (traditional cacao agroforests and Atlantic Forest fragments), while that of the rodents was negative values (TABLE 1).

Table 1. Morphological parameters of marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests (1) and Atlantic Forest fragments (2) in southern Bahia, Brazil. Values are represented as x ± SD, where x = average and SD = standard deviation. N = number of animals. BW = Body weight (g), BL = Body length (mm) e BCI = Body condition index.

			Morphological parameters x ± SD		
Order	**Habitat**	**N**	**BW (g)**	**BL (mm)**	**BCI**
Didelphimorphia	1	17	55.15 ± 76.23	122.82 ± 40.13	0.0064 ± 22.42
	2	10	86.95 ± 143.95	137.60 ± 62.57	0.0006 ± 38.07
Rodentia	1	16	65.13 ± 52.98	124.03 ± 41,34	-0.0043 ± 16.45
	2	7	67.71 ± 22.55	125.57 ± 19,79	-0.0009 ± 4.46

The Atlantic Forest Oryzomys *H. seuanezi* from Atlantic Forest fragments presented body weight and body length lower than those from traditional cacao agroforests. *H. seuanezi* from Atlantic Forest fragments, showed negative BCI, while those from traditional cacao agroforests, has positive ones (TABLE 2). For *M. murina*, both body weight and body length were higher in Atlantic Forest fragments. *M. murina* from Atlantic Forest fragments, showed positive BCI, while those from traditional cacao agroforests, presented BCI with negative values (TABLE 2).

Table 2. Morphological parameters of two species of small mammals with sample size > 5 (*H. seuanezi* and *M. murina*) from the Traditional cacao agroforests (1) and Atlantic Forest fragments (2) in southern Bahia, Brazil. Values are represented as x ± SD, where x = average and SD = standard deviation. N = number of animals. BW = Body weight (g), BL = Body length (mm) e BCI = Body condition index.

			Morphological parameters x ± SD		
Species	**Habitat**	**N**	**BW (g)**	**BL (mm)**	**BCI**
Hylaeamys seuanezi Weksler, Geise & Cerqueira, 1999	1	5	70.90 ± 43.63	133.10 ± 35.75	0.0051 ± 13.84
	2	5	63.60 ± 21.13	121.20 ± 19.18	-0.0049 ± 4.50
Marmosa murina Linnaeus, 1758	1	10	27.30 ± 10.36	108.50 ± 11.54	-0.0047 ± 9.92
	2	7	35.21 ± 16.61	116 ± 21	0.0035 ± 8.20

Lead (Pb) was detected in all hair samples (23.95 ± 16 mg/Kg), regardless species or of habitat, followed by Manganese (Mn) and Copper (Cu), being found in 42 hair samples (1.24 ± 2.27 mg/Kg) and 44 hair samples (1.08 ± 1.54 mg/Kg), respectively. Nickel (Ni) and Cadmium (Cd) were detected in only a few samples: Ni in six hair samples (4.45 ± 4.38 mg/Kg) and Cd in only one hair sample (1.42 mg/Kg). Chromium (Cr) was not detected in concentrations in hair samples of small mammals.

Small mammals from both habitats showed heavy metals concentrations in their hair, evidencing that both study habitats are contaminated (FIGURE 2). Lead (Pb), nickel (Ni), manganese (Mn) and copper (Cu) were detected in both habitats (**traditional cacao agroforests:** Pb (n = 34) - 26.42 ± 16.42 mg/Kg; Ni (n = 5) - 5.31 ± 4.3 mg/Kg; Mn (n = 29) - 1.64 ± 2.63 mg/Kg; Cu (n = 27) - 1.13 ± 1.15 mg/Kg; **Atlantic Forest fragments:** Pb (n = 18) - 19.28 ± 14.46 mg/Kg; Ni (n = 1) – 0.18 mg/Kg; Mn (n = 13) - 0.34 ± 0.40 mg/Kg; Cu (n = 17) - 1 ± 2.05 mg/Kg), but the individuals of the traditional cacao agroforests showed high concentrations of all these heavy metals in comparison to individuals from the Atlantic Forest fragments. Cadmium (Cd) was detected in only one small mammal (1.42 mg/Kg), that were from traditional cacao agroforests. Mean concentrations of heavy metal lead (Pb) and copper (Cu) varied significantly among the habitats (traditional cacao agroforests and Atlantic Forest fragments) (Pb - p = **0.047** and Cu - p = **0.031**) (Supplementary Material – TABLE 3). In addition, regarding the mean concentrations of heavy metal, lead (Pb) was the only metal that varied significantly among the municipalities (Belmonte, Ilhéus and Una) (p = **0.050**) (Supplementary Material – FIGURE 1 and TABLE 3).

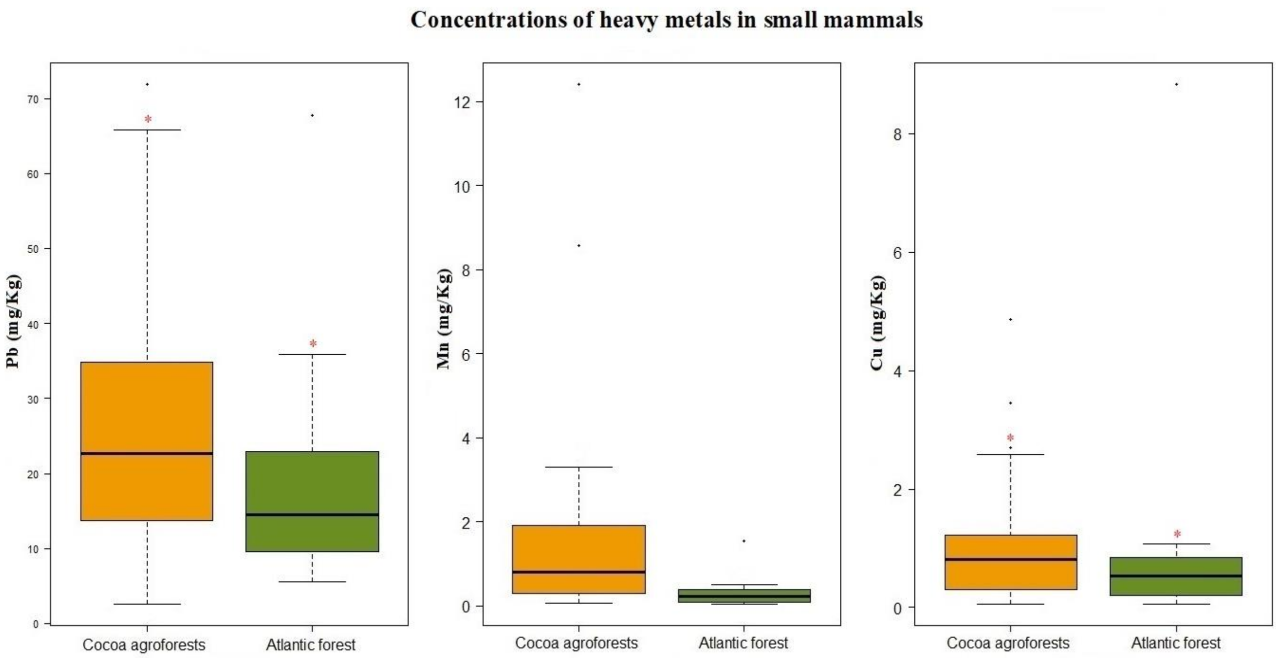


Figure 2. Concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in small mammals from the Traditional cacao agroforests (orange) and Atlantic Forest fragments (green) in southern Bahia, Brazil. * = Indicates a significant difference in lead (Pb) and copper (Cu)

concentrations between habitats (Pb: p = **0.047**; Mn: p = 0.267; Cu: p = **0.031**, ANOVA rank test).

Among the two-order analyzed, Didelphimorphia and Rodentia, both showed heavy metals concentrations (Supplementary Material – FIGURE 2). Lead (Pb), nickel (Ni), manganese (Mn) and copper (Cu) were detected in both order (**Didelphimorphia:** Pb (n = 28) - 28.66 ± 18.73 mg/Kg; Ni (n = 4) - 2.89 ± 4.66 mg/Kg; Mn (n = 21) - 1.01 ± 1.84 mg/Kg; Cu (n = 24) - 1.31 ± 1.81 mg/Kg; **Rodentia:** Pb (n = 24) - 18.44 ± 9.85 mg/Kg; Ni (n = 2) - 7.57 ± 1.41 mg/Kg; Mn (n = 21) - 1.47 ± 2.66 mg/Kg; Cu (n = 20) - 0.80 ± 1.12 mg/Kg). Mean concentrations of lead (Pb) varied significantly among the order (p = **0.038**) (Supplementary Material – TABLE 4). Marsupials showed high concentrations of lead in comparison to rodents. Cadmium (Cd) was detected only in marsupials (1.42 mg/Kg).

Marsupials and rodents from both habitats showed lead (Pb) manganese (Mn) and copper (Cu) concentrations in their hair, evidencing that both study habitats are contaminated (FIGURE 3 and TABLE 3). Marsupials of the traditional cacao agroforests showed high concentrations of lead and manganese in comparison to them of the Atlantic Forest fragments. Rodents of the traditional cacao agroforests showed high concentrations of all these heavy metals in comparison to rodents from the Atlantic Forest fragments. In the order, mean concentrations of lead (Pb) in marsupials and rodents varied significantly among the habitats (traditional cacao agroforests and Atlantic Forest fragments) (p = **0.056**) (Supplementary Material – TABLE 5). Marsupials of the traditional cacao agroforests showed high concentrations only for lead in comparison to rodents of the traditional cacao agroforests, and marsupials of the Atlantic Forest fragments showed high concentrations of lead, manganese and copper in comparison to rodents from the Atlantic Forest fragments. In relation to the other heavy metals that were detected > 10 samples: Nickel (Ni) was detected in marsupials from both habitats (traditional cacao agroforests: Ni (n = 3) - 3.80 ± 5.25 mg/Kg); Atlantic Forest fragments: Ni (n = 1) - 0.18 mg/Kg). In rodents, nickel was only detected in traditional cacao agroforests (n = 2) - 7.57 ± 1.41 mg/Kg). Cadmium (Cd) was detected in only one marsupial from traditional cacao agroforests (1.42 mg/Kg). In addition, regarding the mean concentrations of heavy metals in marsupials and rodents occurring independently of the municipalities (Belmonte, Ilhéus and Una) (Supplementary Material – TABLE 5).

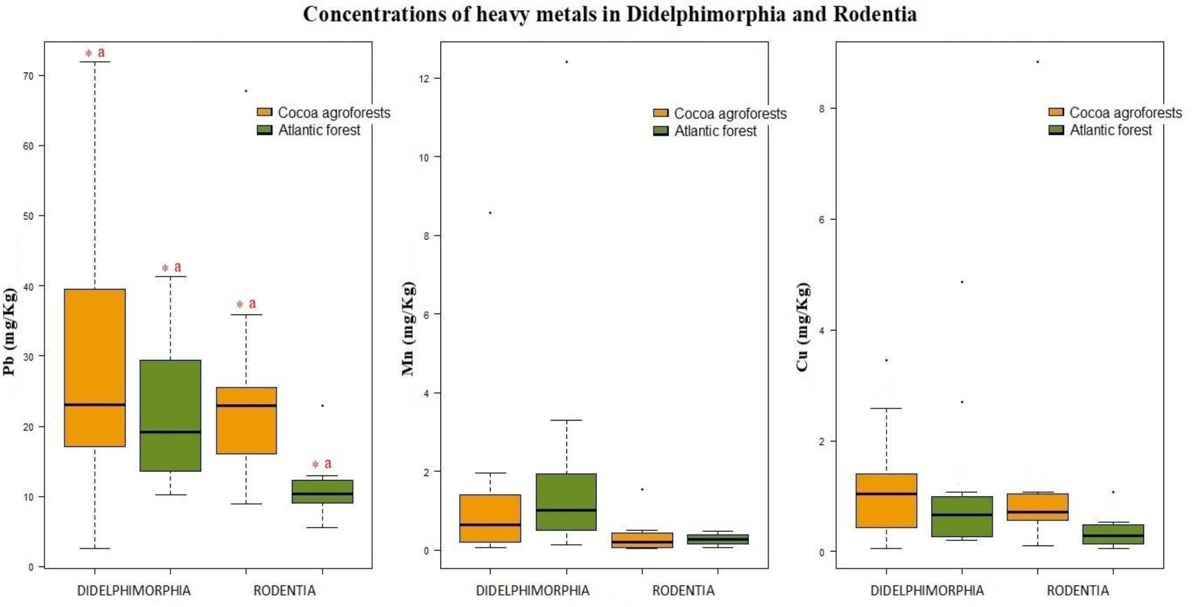


Figure 3. Concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests (orange) and Atlantic Forest fragments (green) in southern Bahia, Brazil. * = Indicates a significant difference in lead (Pb) concentration between order of small mammals (Pb: p = **0.038**; Mn: p = 0.296; Cu: p = 0.094, Kruskal-Wallis test). a = Indicates a significant difference in lead (Pb) concentration between habitats (Pb: p = **0.059**; Mn: p = 0.647; Cu: p = 0.136, ANOVA rank test).

Table 3. Concentration of heavy metals (mg/Kg) in hair of marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests (1) and Atlantic Forest fragments (2) in southern Bahia, Brazil. Values are represented as x ± SD (min. - max.), where x = average, SD = standard deviation, min.= minimum value and max. = maximum value. N = number of animals.

			Concentration of heavy metals, mg/Kg x ± SD		
Order	**Habitat**	**N**	**Pb**	**Mn**	**Cu**
Didelphimorphia	1	18	30.41 ± 20.03 (2.53 – 71.90)	1.32 ± 2.19 (0.05 – 8.57)	1.16 ± 0.99 (0.05 – 3.45)
	2	10	25.52 ± 16.64 (8.94 – 67.73)	0.40 ± 0.54 (0.03 – 1.55)	1.56 ± 2.75 (0.10 – 8.84)
Rodentia	1	16	21.93 ± 9.88 (10.23 – 41.27)	1.95 ± 3.04 (0.12 – 12.41)	1.09 ± 1.37 (0.20 – 4.86)
	2	8	11.48 ± 5.16 (5.54 – 22.99)	0.28 ± 0.16 (0.06 – 0.48)	0.36 ± 0.33 (0.05 – 1.07)

The Atlantic Forest Oryzomys (*H. seuanezi*) showed only concentrations of Pb, Mn and Cu in their hair samples, it's worth noting that individuals of this species exhibited high levels of lead in their hair, when compared with the other metals (Pb = 16.87 ± 7.90 mg/Kg, Mn = 0.5 ± 0.49 mg/Kg and Cu = 0.3 ± 0.17 mg/Kg). Mean concentrations of lead (Pb) varied significantly among the habitats (traditional cacao agroforests and Atlantic Forest fragments) (p = **0.031**) (Supplementary Material – TABLE 6), individuals of the traditional cacao agroforests showed high concentrations of heavy metals in comparison to individuals from the Atlantic Forest fragments (TABLE 4). The Linnaeus's Mouse Opossum (*M. murina*) showed all heavy metals concentrations in their hair, except chromium. Lead (Pb), manganese (Mn) and copper (Cu) concentrations from traditional cacao agroforests and Atlantic Forest fragments were shown in Supplementary Material – FIGURE 3 and TABLE 4. In relation to the other heavy metals: Nickel (Ni) was detected in both habitats (**traditional cacao agroforests:** 5.26 ± 6.51 mg/Kg; **Atlantic Forest fragments:** 0.18 mg/Kg). Cadmium (Cd) was detected only in traditional cacao agroforests (1.42 mg/Kg). The bioaccumulation of heavy metals in *M. murina* occurred independently of the habitats (Supplementary Material – TABLE 7).

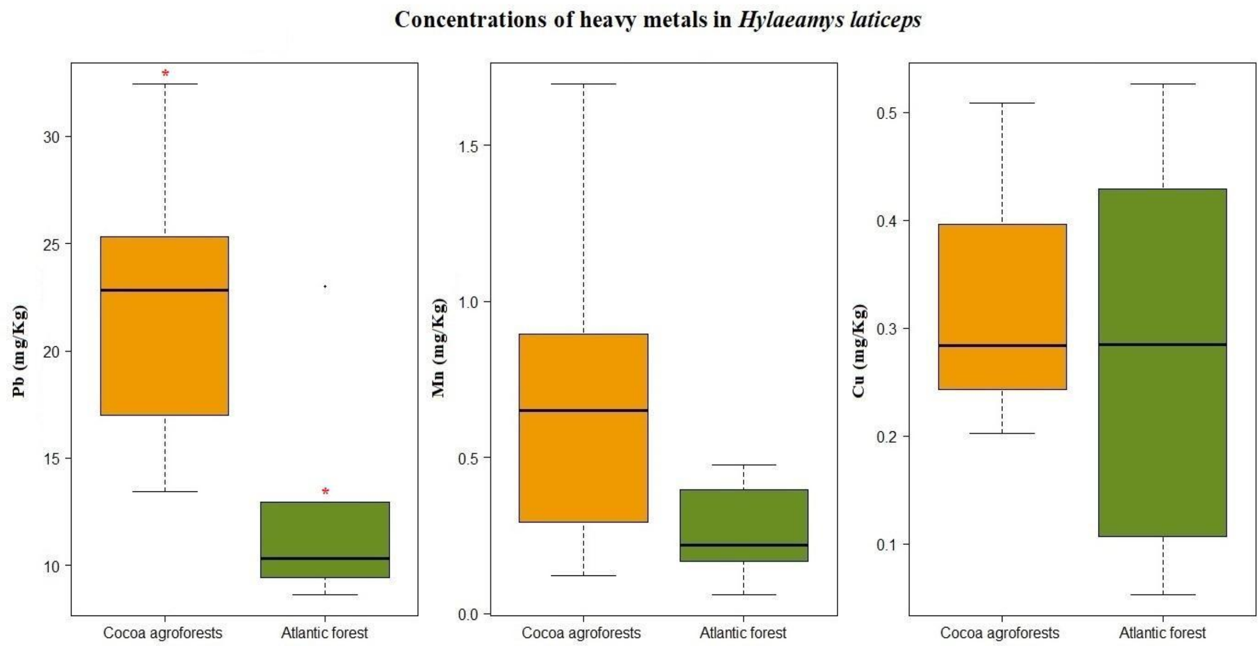


Figure 4. Concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in Atlantic Forest Oryzomys (*Hylaeamys seuanezi*, Rodentia) from the Traditional cacao agroforests (orange) and Atlantic Forest fragments (green) in southern Bahia, Brazil. * = Indicates a significant difference in lead (Pb) concentration between habitats in the hair of *H. seuanezi* (Pb: p = **0.031**; Mn: p = 0.141; Cu: p = 0.699, ANOVA test).

Table 4. Concentration of heavy metals (mg/Kg) in hair of two species of small mammals with sample size > 5 (*Hylaeamys seuanezi* and *Marmosa murina*) from the Traditional cacao agroforests (1) and Atlantic Forest fragments (2) in southern Bahia, Brazil. Values are represented as x ± SD (min. - max.), where x = average, SD = standard deviation, min.= minimum value and max. = maximum value. N = number of animals.

			Concentration of heavy metals, mg/Kg x ± SD		
Species	**Habitat**	**N**	**Pb**	**Mn**	**Cu**
Hylaeamys seuanezi Weksler, Geise & Cerqueira, 1999	1	5	22.20 ± 7.39 (13.45 – 32.43)	0.73 ± 0.62 (0.12 – 1.70)	0.33 ± 0.16 (0.20 – 0.51)
	2	6	12.43 ± 5.39 (8.62 – 22.99)	0.26 ± 0.17 (0.06 – 0.48)	0.28 ± 0.19 (0.05 – 0.53)
Marmosa murina Linnaeus, 1758	1	11	27.83 ± 15.76 (9.36 – 65.78)	1.77 ± 2.83 (0.12 – 8.57)	1.47 ± 1.16 (0.05 – 3.45)
	2	7	28.24 ± 18.97 (11.60 – 67.73)	0.48 ± 0.62 (0.06 – 1.55)	1.89 ± 3.42 (0.10 – 8.84)

Although the other species of small mammals, all its analyzed hair samples showed concentrations of at least one heavy metal (see Supplementary Material – TABLE 8). Three accumulation pathways were identified: lead – manganese, nickel – cadmium and copper accumulation pathway (FIGURE 5). The Pearson Correlation Test did not show a significant correlation between lead, manganese and copper (Supplementary Material – TABLE 9).

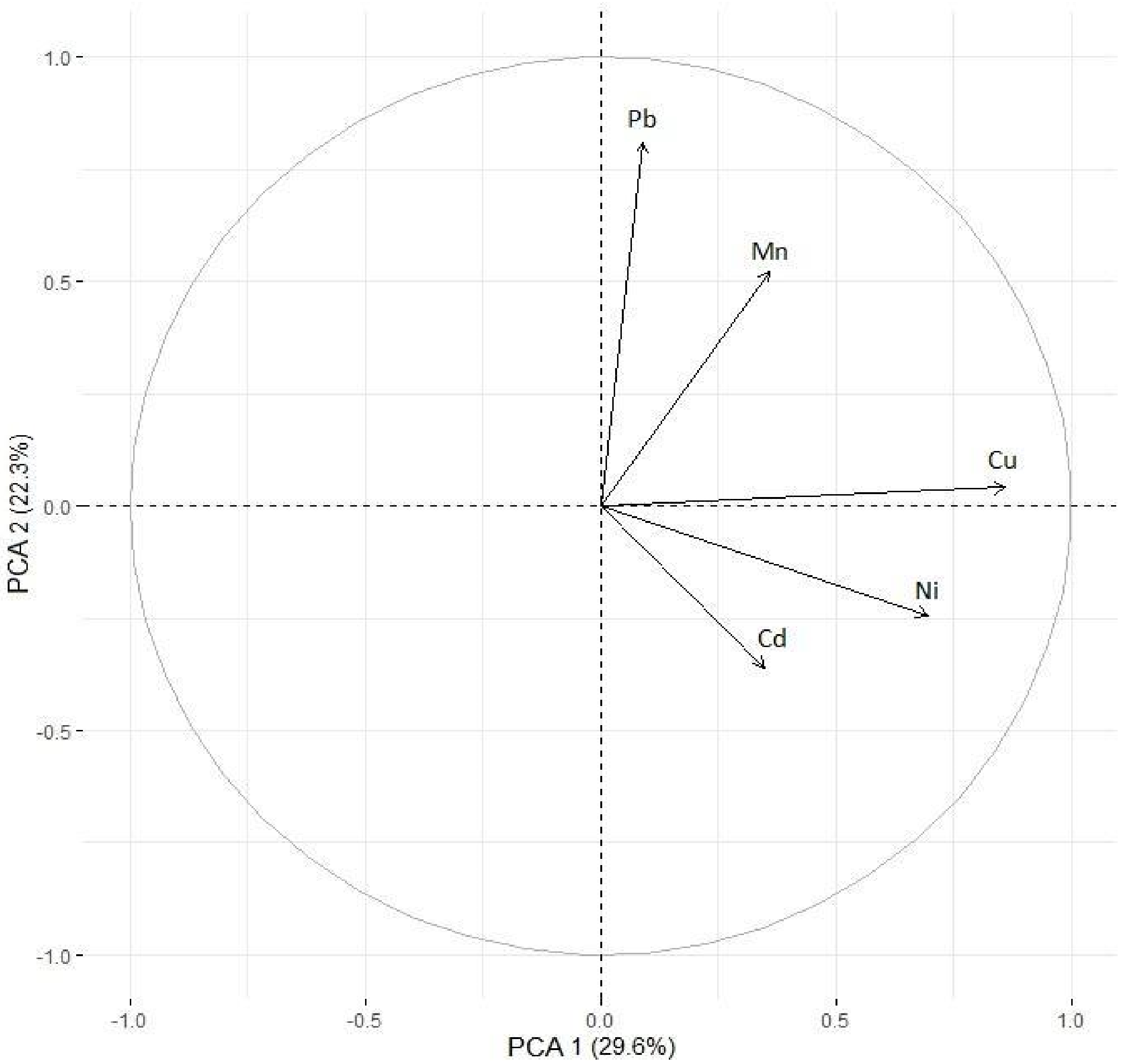


Figure 5. Principal Component Analysis (PCA) of Nickel (Ni), Lead (Pb), Cadmium (Cd), Chromium (Cr), Manganese (Mn) and Copper (Cu) concentrations (mg/Kg) in all small mammals captured from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil.

Among the two analyzed order, marsupials and rodents, there is a significant effect of body length on the weight of these animals. The habitat does not affect the BCI of marsupials and rodents (Supplementary Material – TABLE 10). For *H. seuanezi*, there is no effect of body length on the weight of these animals, however the interaction between body length and habitat significantly affects the weight of these animals. For *M. murina*, however, there is a significant effect of body length on the weight of these animals, but the habitat does not affect the BCI of *M. murina* (Supplementary Material – TABLE 11).

The concentration of lead, manganese and copper occurred independently of the body condition index (BCI) and habitats (traditional cacao agroforests and Atlantic Forest fragments) for marsupials and rodents (Supplementary Material – TABLE 12).

In relation two species of small mammals (*H. seuanezi* and *M. murina*) that occurred in both habitats, the concentration of lead and copper occurred significantly between the body condition index (BCI) and habitat (Pb – p = **0.044** and Cu – p = **0.033**) only for *H. seuanezi* (Supplementary Material – TABLE 13).

Discussion

To our knowledge, studies of heavy metal levels in small mammals' hair in the Neotropics have not previously been performed. This is the first study to evaluate heavy metal contamination in small mammals in traditional cacao agroforests and Atlantic Forest fragments in Brazil, thus providing important data that indicate the presence of heavy metals in the hair of the Neotropical small terrestrial mammal's species. We found that the habitats seemed to influence the bioaccumulation since we found higher concentrations of Pb and Cu in hair of small mammals from traditional cacao agroforests in comparison the concentrations observed in hair of small mammals from Atlantic Forest fragments, corroborating our initial hypothesis. Neither habitat nor bioaccumulation of heavy metals seem to have affected body condition in small mammals because we found no significant differences in any of those variables.

The presence of higher concentrations of heavy metals in the hair of small mammals from traditional cacao agroforests is possibly due to the application of agrochemicals (copper-based fungicides and phosphate-based fertilizers) to control diseases and increase cacao productivity, like Funguran^{-OH}, Champ DP, Ridomil Gold 66 WP, Copper Nordox 50 WP and 75 WP, Kocide 101 and 2000, Ultimax plus, Phostoxin, Bordeaux mixture, Perenox, Lindane, Capsitox, Diazinon, Propouxur, Basudin 600 EC, Maneb and Mancozeb (Mokwunye et al., 2012; Muoghalu and Odiwe, 2011), which have levels of heavy metals in their composition (Gimeno-García et al.,1996; Ju et al., 2007; López-Carnelo et al., 1997), thus representing serious environmental and health risks for rural farmers, since agrochemical applications are carried out without any control and safety measures. The lower values of heavy metals observed in the Atlantic Forest fragments can be attributed to the fact that the habitat is free from the application of chemical pesticides, since has no agricultural practices. The result was in line with the work of Ekpo (2017), who reported the higher concentration of heavy metals (Fe, Zn, Cu, Pb, Cr, Cd and Ni) in soil obtained from fungicide-treated cacao plantations compared to the control location (Mfanosing) in the Akamkpa Local Government Area of Cross River State Nigeria. The bioaccumulation of heavy metals in the hair of small mammals in both habitats (traditional cacao agroforests and Atlantic Forest fragments) showed significant differences ($p < 0.05$) for lead and copper.

The concentrations of the lead in the hair of small mammals from traditional cacao agroforests and Atlantic Forest fragments were higher than the World Health Organization - WHO (2001) permissible limits. While the concentrations of the other heavy metals (Ni, Cd, Cu and Mn) recorded in the hair of small mammals from traditional cacao agroforests and Atlantic Forest fragments were within the permissible limits. WHO permissible limits for heavy metals in soil was Ni – 0.5-6.5 mg/Kg, Pb – 0.05-0.1 mg/kg, Cd – 0.005-0.01 mg/ kg and Cu – 0.05-1.5 mg/kg. In line with our results, Arham et al. (2017) reported the heavy metals Pb and Cd in cacao plantation soils of East Kolaka, Indonesia, exceeded the WHO permissible, and Ni, Cu, and Zn are still within the permissible limits. The lead concentration in the hair of small mammals from Atlantic Forest fragments was higher than that found by Brait et al. (2009) on maned wolf (*Chrysocyon brachyurus*), crab-eating Fox (*Cerdocyon thous*) and ocelot (*Leopardus pardalis*) hair in Parque Nacional das Emas, Brazil. In this way, we must have a close look at the concentrations of lead obtained in the hair of small mammals from these areas, since lead is considered a non-essential heavy metal, which is among the most toxic and dangerous metals in the world (Engbersen et al., 2019). Pb is toxic even at low concentrations, thus becoming a global concern for both environmental and human health.

Lead levels have dramatically increased in the environment due to human activities. The intensification of urbanization, agricultural practices, such as the application of fertilizers, pesticides containing Pb in their composition and soil additives, industries and higher automobile traffic, which results in the release of automobile exhaust gasses originating from the anti-knock agent added to gasoline, are examples. From anthropogenic sources of lead (Alkimin-Filho, 2011; Matte, 2003), resulting in contamination of soil biota, plants, animals and even man (Mariam et al., 2004). The increasing exposure to lead leads to serious environmental damage, since Pb can enter the food chain. The presence of high concentrations of Pb in the soil results in their uptake by plants, leading to possible bioaccumulation in animals that feed on them, thus harming the well-being of the environment and reducing the quality of life (Aikpokpodian et al., 2012; Albarrcín et al., 2019; Frink, 1996; Ogunlade and Agbeniyi, 2011). In addition, exposure to lead results in damage to the health of animals and humans, causing serious effects on the cardiovascular, renal, immunological, neurological system, as well as behavioral damage, cancer and even death (Agency for Toxic Substances and Disease Registry – ATSDR, 2007; Carrington and Bolger, 1992; European Food Safety Authority – EFSA, 2012; Lopes, 2009; World Health Organization – WHO, 2011). Thus, the potential toxicity of Pb to soil, animals and humans requires adequate attention (Onianwa et al., 1999).

Our results showed differences in lead (Pb) bioaccumulation regarding the order of small mammals. Marsupials had higher lead concentrations compared to rodents, not agreeing with our initial hypothesis. This could be associated with eating habits and habitat use. Marsupials are arboreal with an insectivorous-omnivore diet (Paglia et al., 2012; Santori et al., 1995). These animals mainly consume arthropods, such as beetle and ants (Carvalho et al., 2005), occasionally fruits and small vertebrates. They usually foraging in close contact with the leaf litter (Freitas et al., 1997; Caceres and Monteiro-Filho, 2001), where some of their main food items, such as insects, are more accessible. Thus, their food preferences may result in a greater likelihood of contact with a contaminated food (Hunsaker and Shupe, 1977), since insects accumulate high concentrations of heavy metals, mainly Pb, in their bodies and at higher rates in the exoskeleton (Butovsky et al., 1999; Roberts and Johnson, 1978). While rodents, although terrestrial, living in direct and constant contact with the soil (Khazaee et al., 2015), have a frugivorous/granivore-omnivorous diet (Paglia et al., 2012), relying more on fruits, leaves and nectar. In addition, marsupials are considered ideal species for studying the impacts of anthropogenic pollution, since, due to their lifestyle, they are often found in urban and peri-urban areas (Harmon et al., 2005), thus having an intimate contact with countless pollutants (Shore and Rattner, 2001), including heavy metals.

Additionally in this study, we analyzed separately two species of small mammals, *Hylaeamys seuanezi* and *Marmosa murin*a, because with increasing use of chemicals, including pesticides, there is a need to develop zoobioindicators of exposure. Both species are relatively common and available in the studied areas, have a relatively large body size and an appropriate life expectancy to measure recent exposure to contaminants (National Research Council - NRC, 1991). In our results, we saw that these two species accumulate heavy metals in their hair, indicating that they are probably species with a good biological response for the evaluation of contaminants, since the accumulated levels were sufficient to be measured. It is suggested that hair of this small mammal's species can be a useful bioindicator of local contamination. However, heavy metal levels in Neotropical small terrestrial mammals are generally not examined, making comparisons difficult. In addition, there are no small mammal data (no tissue) from traditional cacao agroforests and Atlantic Forest fragments or from other areas of Brazil.

Conclusions

Our results indicate that possibly both habitats are contaminated by lead, since this heavy metal is above the limit allowed for soils. This is the first study to evaluate heavy metal contamination in small mammals in traditional cacao agroforests and Atlantic Forest fragments in Brazil, thus providing important data that indicate the presence of heavy metals in the hair of the small mammal species studied, and consequently in the environment where they are found. This study can serve as a guide for future studies, especially in Brazil and South America, where there is limited data on monitoring heavy metal concentrations in samples from Neotropical small terrestrial mammals. In the future, studies on the relationship between hair concentration and concentrations in internal organs, such as the liver and kidney, and in soil for non-essential and essential heavy metals can be carried out, in addition to monitoring the same animals temporally to provide repeated measures on the seasonal fluctuations in the concentration of metals in these animals. Other contaminants must also be evaluated to profile heavy metal contamination in Brazil and the Neotropics.

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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.

Ethical statement

The captured animals were managed under authorizations by SISBIO / ICMBio: 69688-4 and CEUA – UESC: 005/2019. All capture and handling protocol followed the guidelines of the American Society of Mammals.

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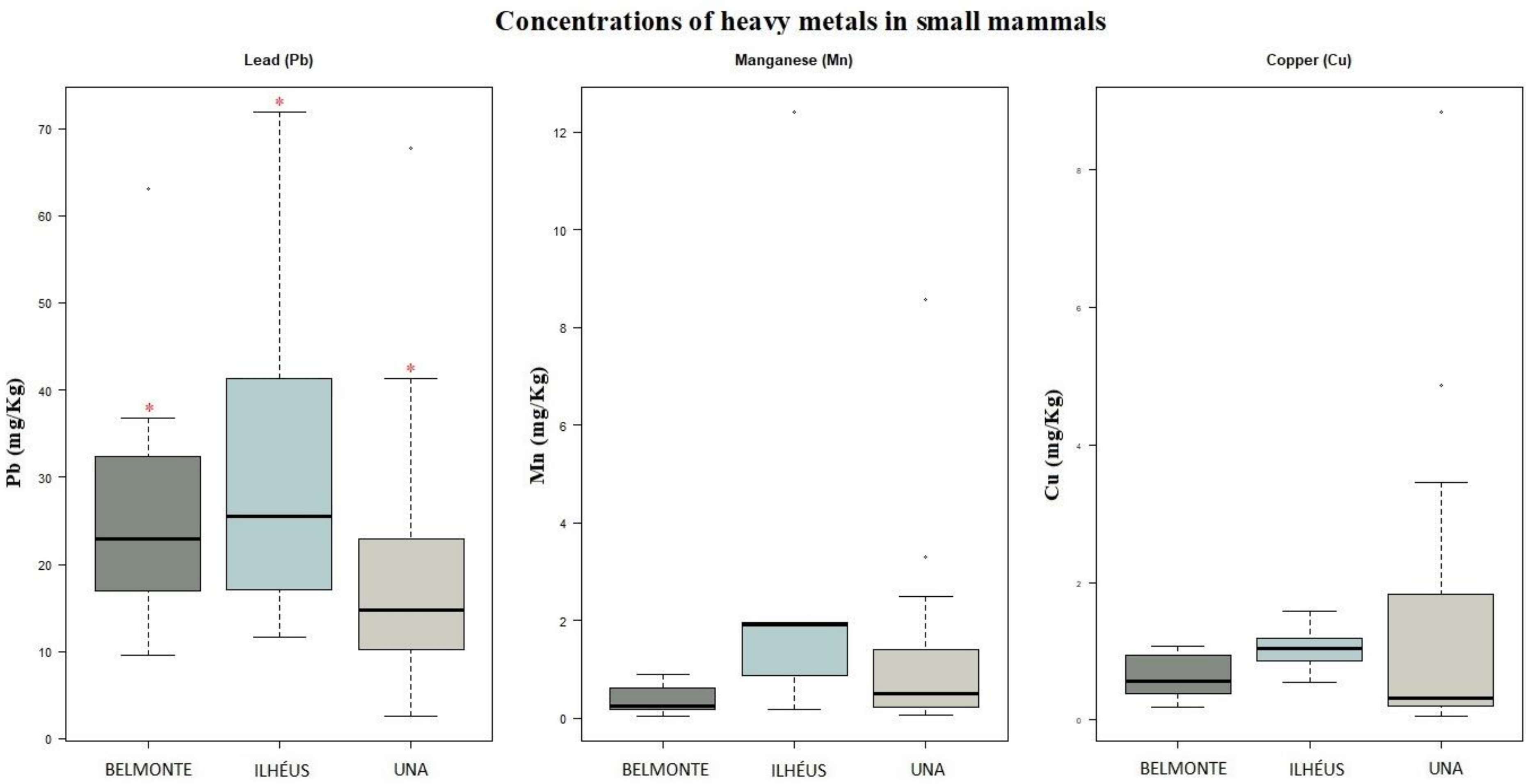
Appendix A. Supplementary material

Figure’s caption

Figure 1. Concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in small mammals from Belmonte, Ilhéus and Una municipalities, in southern Bahia, Brazil. * = Indicates a significant difference in lead (Pb) concentration between municipalities (Pb: p = **0.050**; Mn: p = 0.303; Cu: p = 0.302, ANOVA rank test)

Figure 2. Concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in marsupials (Didelphimorphia) and rodents (Rodentia) in southern Bahia, Brazil. * = Indicates a significant difference in lead (Pb) concentration between order of small mammals (Pb: p = **0.038**; Mn: p = 0.296; Cu: p = 0.094, Kruskal-Wallis test).

Figure 3. Concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in Linnaeus's Mouse Opossum (*Marmosa murina*, Didelphimorphia) from the Traditional cacao agroforests (orange) and Atlantic Forest fragments (green) in southern Bahia, Brazil.



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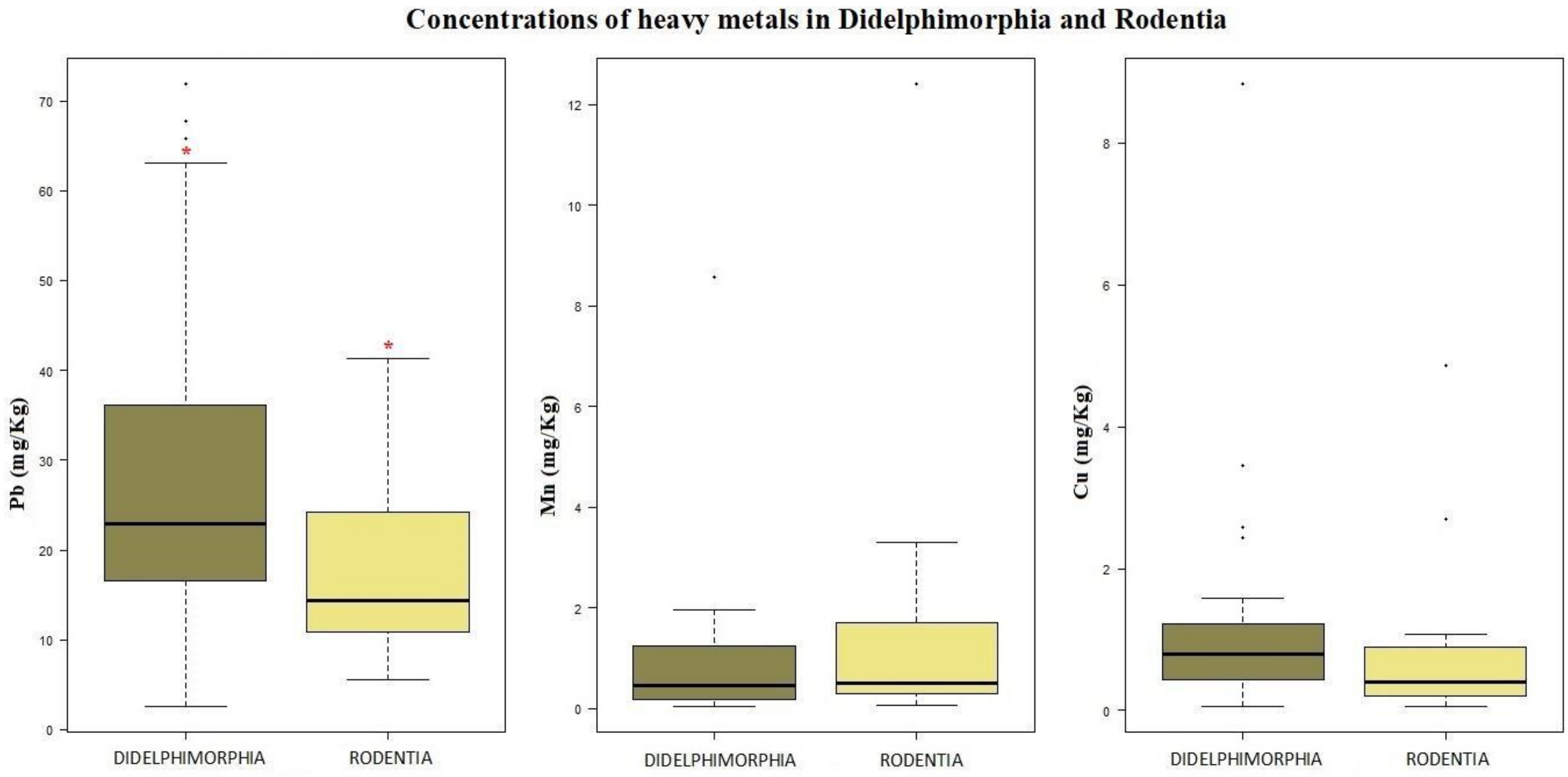

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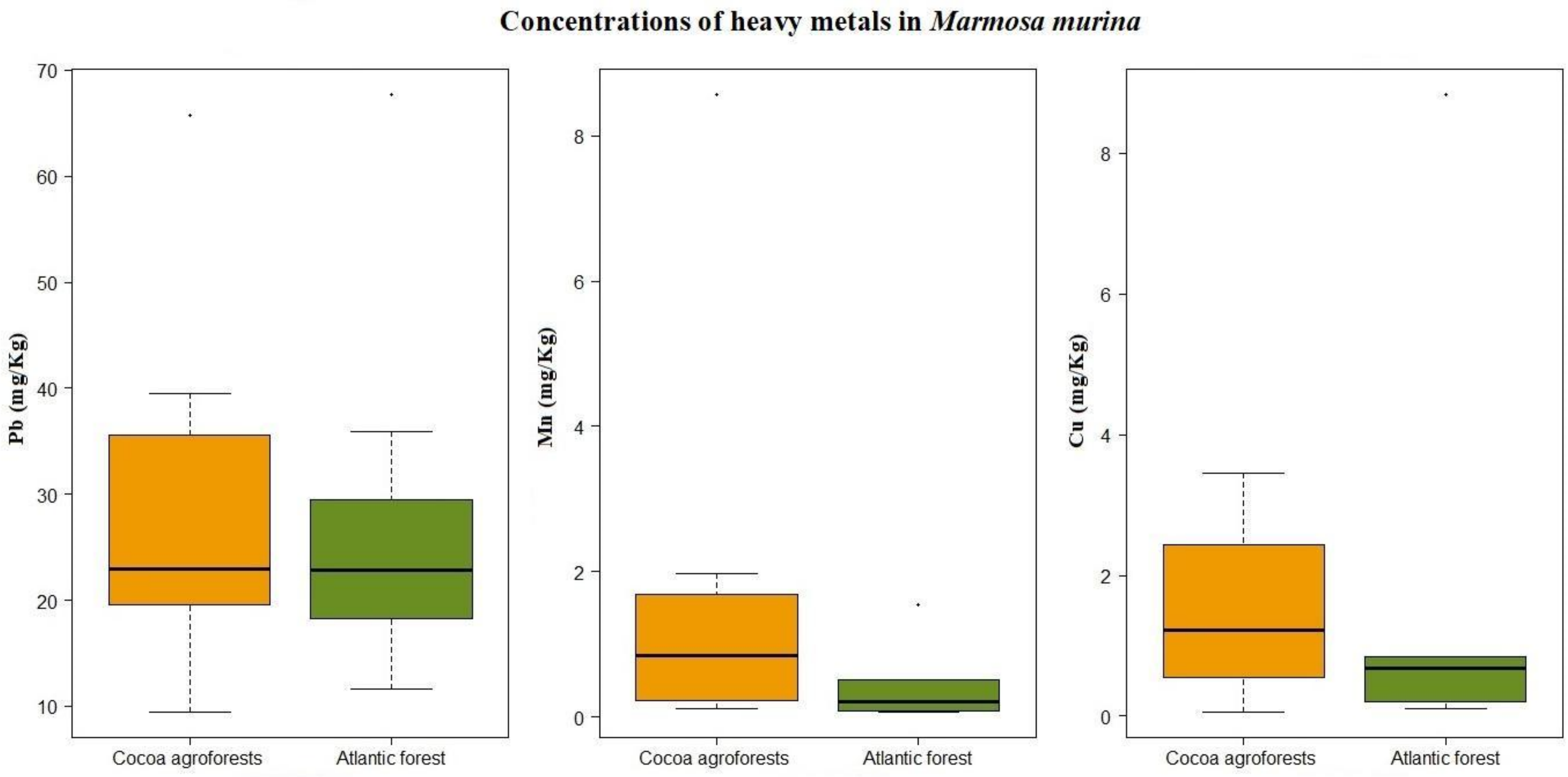

Figure 3. Concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in Linnaeus's Mouse Opossum (*Marmosa murina*, Didelphimorphia) from the Traditional cacao agroforests (orange) and Atlantic Forest fragments (green) in southern Bahia, Brazil.

Table's caption

Table 1. Number of individuals of small mammals captured in Traditional cacao agroforests and Atlantic Forest fragments southern Bahia, Brazil.

Table 2. Kruskal-Wallis test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in hair in all small mammals species captured in southern Bahia, Brazil. P values in bold represent significant values.

Table 3. ANOVA rank test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in all small mammals captured from the Traditional cacao agroforests and Atlantic Forest fragments, in Belmonte, Ilhéus and Una municipalities, in southern Bahia, Brazil. P values in bold represent significant values.

Table 4. Kruskal-Wallis test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in marsupials (Didelphimorphia) and rodents (Rodentia) in southern Bahia, Brazil. P values in bold represent significant values.

Table 5. ANOVA rank test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests and Atlantic Forest fragments, in Belmonte, Ilhéus and Una municipalities, in southern Bahia, Brazil. P values in bold represent significant values.

Table 6. ANOVA test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in Atlantic Forest Oryzomys (*Hylaeamys seuanezi*, Rodentia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Table 7. Kruskal-Wallis test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in Linnaeus's Mouse Opossum (*Marmosa murina*, Didelphimorphia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Table 8. Concentration of heavy metals (Ni = nickel, Pb = lead, Cd = cadmium, Cr = chromium, Mn = manganese and Cu = copper) (mg/Kg) in hair of species of small mammals with sample size < 5 from the Traditional cacao agroforests (1) and Atlantic Forest fragments (2) in southern Bahia, Brazil. Values are represented as $x \pm SD$, where x = average and SD = standard deviation. N = number of animals, - = undetected values of concentration of heavy metals.

Table 9. Pearson's Correlation Tests among heavy metals (Pb = lead; Mn = manganese and Cu = copper) in all small mammals captured from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Table 10. ANCOVA results table for body condition index (BCI) in marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Table 11. ANCOVA results table for body condition index (BCI) in Atlantic Forest Oryzomys (*Hylaeamys seuanezi*, Rodentia) and Linnaeus's Mouse Opossum (*Marmosa murina*, Didelphimorphia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Table 12. PERMANOVA results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) and body condition index (BCI) in marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; F: F value by permutation and p value: p-values based on more than 9000 permutations (the lowest possible p-value is 0.0001). P values in bold represent significant values.

Table 13. PERMANOVA results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) and body condition index (BCI) in Atlantic Forest Oryzomys (*Hylaeamys seuanezi*, Rodentia) and Linnaeus's Mouse Opossum (*Marmosa murina*, Didelphimorphia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; F: F value by permutation and p value: p-values based on more than 9000 permutations (the lowest possible p-value is 0.0001). P values in bold represent significant values.

Table 1. Number of individuals of small mammals captured in Traditional cacao agroforests and Atlantic Forest fragments southern Bahia, Brazil.

Species	Traditional cacao agroforests	Atlantic Forest remnants	N
Rodentia			
Akodon cursor Winge, 1887	3	1	**4**
Guerlinguetus ingrami Thomas, 1901	1	0	**1**
Hylaeamys seuanezi Weksler, Geise & Cerqueira, 1999	5	6	**11**
Oecomys catherinae Thomas, 1909	1	0	**1**
Phyllomys pattoni Emmons, Leite, Kock & Costa, 2002	0	1	**1**
Rattus sp.	1	0	**1**
Rhipidomys mastacalis Lund, 1841	3	0	**3**
Spp.	1	0	**1**
Thaptomys nigrita Lichtenstein, 1830	1	0	**1**
Marsupials			
Didelphis aurita Wied-Neuwied, 1826	1	1	**2**
Marmosa murina Linnaeus, 1758	11	7	**18**
Marmosops incanus Lund, 1841	5	2	**7**
Metachirus nudicaudatus Desmarest, 1817	1	0	**1**
TOTAL	**34**	**18**	**52**

Table 2. Kruskal-Wallis test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in hair in all small mammals species captured in southern Bahia, Brazil. P values in bold represent significant values.

Heavy metals	**Chi-squared**	**df**	**p value**
Pb	27.048	12	**0.007**
Mn	13.199	11	0.280
Cu	19.749	12	0.071

Table 3. ANOVA rank test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in all small mammals captured from the Traditional cacao agroforests and Atlantic Forest fragments, in Belmonte, Ilhéus and Una municipalities, in southern Bahia, Brazil. P values in bold represent significant values.

Lead (Pb)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Habitat	1	832.8	832.76	41.380	**0.047**
Municipalities	2	1282.2	641.10	31.856	**0.050**
Habitat : Municipalities	2	340.7	170.35	0.8465	0.435
Residuals	46	9257.4	201.25		
Manganese (Mn)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Habitat	1	295.8	295.771	12.579	0.267
Municipalities	2	575.1	287.572	12.231	0.303
Habitat : Municipalities	2	26.3	13.171	0.0560	0.945
Residuals	46	10815.7	235.125		
Copper (Cu)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Habitat	1	1065.8	1065.83	49.326	**0.031**
Municipalities	2	529.9	264.94	12.261	0.302
Habitat : Municipalities	2	177.6	88.80	0.4110	0.665
Residuals	46	9939.7	216.08		

Table 4. Kruskal-Wallis test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in marsupials (Didelphimorphia) and rodents (Rodentia) in southern Bahia, Brazil. P values in bold represent significant values.

Heavy metals	Chi-squared	df	p value
Pb	4.3022	1	**0.038**
Mn	1.0899	1	0.296
Cu	2.8006	1	0.094

Table 5. ANOVA rank test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests and Atlantic Forest fragments, in Belmonte, Ilhéus and Una municipalities, in southern Bahia, Brazil. P values in bold represent significant values.

Lead (Pb)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Order	1	988.1	988.08	60.479	**0.018**
Habitat	1	879.2	879.19	53.814	**0.025**
Municipalities	2	1202.9	601.43	36.813	**0.034**
Order : Habitat	1	628.7	628.72	38.484	**0.056**
Order : Municipalities	2	680.9	340.45	20.839	0.137
Habitat : Municipalities	2	217.8	108.90	0.6666	0.519
Order : Habitat: Municipalities	2	580.5	290.24	17.765	0.182
Residuals	40	6535.0	163.37		
Manganese (Mn)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Order	1	0.1	0.08	0.0003	0.985
Habitat	1	295.7	295.72	12.004	0.279
Municipalities	2	582.6	291.29	11.824	0.317
Order : Habitat	1	52.4	52.39	0.2127	0.647
Order : Municipalities	2	219.4	109.70	0.4453	0.643
Habitat : Municipalities	2	34.0	17.02	0.0691	0.933
Order : Habitat: Municipalities	2	674.9	337.47	13.699	0.265
Residuals	40	9853.8	246.35		
Copper (Cu)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Order	1	297.5	297.45	13.567	0.251
Habitat	1	1094.8	1094.79	49.934	**0.031**
Municipalities	2	543.1	271.53	12.385	0.300
Order : Habitat	1	505.4	505.44	23.053	0.136
Order : Municipalities	2	190.8	95.41	0.4352	0.650
Habitat : Municipalities	2	188.9	94.45	0.4308	0.653
Order : Habitat: Municipalities	2	122.7	61.35	0.2798	0.757
Residuals	40	8769.8	219.25		

Table 6. ANOVA test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in Atlantic Forest Oryzomys (*Hylaeamys seuanezi*, Rodentia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Lead (Pb)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Habitat	1	260.2	260.24	6.436	**0.031**
Residuals	9	363.9	40.44		
Manganese (Mn)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Habitat	1	0.5473	0.5473	2.665	0.141
Residuals	8	16.431	0.2054		
Copper (Cu)					
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Habitat	1	0.00519	0.00519	0.162	0.699
Residuals	7	0.22389	0.03198		

Table 7. Kruskal-Wallis test results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) in Linnaeus's Mouse Opossum (*Marmosa murina*, Didelphimorphia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Heavy metals	**Chi-squared**	**df**	**p value**
Pb	0.051265	1	0.820
Mn	17.357	1	0.187
Cu	0.5	1	0.479

Table 8. Concentration of heavy metals (Nickel (Ni), Lead (Pb), Cadmium (Cd), Chromium (Cr), Manganese (Mn) and Copper (Cu) (mg/Kg) in hair of species of small mammals with sample size < 5 from the Traditional cacao agroforests (1) and Atlantic Forest fragments (2) in southern Bahia, Brazil. Values are represented as x ± SD, where x = average and SD = standard deviation. N = number of animals, - = undetected values of concentration of heavy metals.

			Concentration of heavy metals, mg/Kg x ± SD					
Species	**Habitat**	**N**	**Ni**	**Pb**	**Cd**	**Cr**	**Mn**	**Cu**
Akodon cursor	1	3	8.56 *****	13.25 ± 3.47	-	-	5.39 ± 6.11	1.45 ± 1.08
	2	1	-	11.69	-	-	0.34	1.07
Didelphis aurita	1	1	0.88	10.82	-	-	0.80	0.33
	2	1	-	8.94	-	-	0.36	0.56
Guerlinguetus ingrami	1	1	-	21.29	-	-	0.18	0.26
	2	0	-	-	-	-	-	-
Marmosops incanus	1	5	-	45.58 ± 22.09	-	-	0.85 ± 0.76 [a]	0.90 ± 0.27 [a]
	2	2	-	24.27 ± 1.84	0.03 *****	-	-	1.06 ± 0.03
Spp.	1	1	-	10.60	-	-	0.50	0.21
	2	0	-	-	-	-	-	-
Oecomys catherinae	1	1	-	13.75	0.50	-	-	1.07
	2	0	-	-	-	-	-	-
Phyllomys pattoni	1	0	-	-	-	-	-	-
	2	1	-	5.54	-	-	-	0.16
Rattus sp.	1	1	-	15.06	-	-	1.19	0.92
	2	0	-	-	-	-	-	-
Rhipidomys mastacalis	1	3	-	37.44 ± 3.53	-	-	1.49 ± 0.67 [b]	0.38 *****
	2	0	-	-	-	-	-	-
Thaptomys nigrita	1	1	6.57	26.26	-	-	3.29	4.86
	2	0	-	-	-	-	-	-

***** = Concentration of heavy metal was detected in only one hair sample.

[a] = Concentration of heavy metal was detected in four hair samples.

[b] = Concentration of heavy metal was detected in two hair samples.

Table 9. Pearson's Correlation Tests among heavy metals (Pb = lead; Mn = manganese and Cu = copper) in all small mammals captured from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Heavy metals	t	df	p value	Pearson's correlation
Pb ~ Mn	0.64355	40	0.523	0.101
Pb ~ Cu	1.1038	42	0.276	0.167
Mn ~ Cu	1.8403	43	0.074	0.305

Table 10. Body condition index (BCI) in marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Didelphimorphia

	Body Weight				
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Body Length	1	257516	257516	258.970	**5.78e-10**
Habitat	1	1571	1571	1.579	0.231
Body Length : Habitat	1	2227	2227	2.240	0.158
Residuals	13	12927	994		

Rodentia

	Body Weight				
	df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Body Length	1	28105	28105	76.646	**5.3e-06**
Habitat	1	5	5	0.013	0.913
Body Length : Habitat	1	13	13	0.036	0.854
Residuals	10	3667	367		

Table 11. Body condition index (BCI) in Atlantic Forest Oryzomys (*Hylaeamys seuanezi*, Rodentia) and Linnaeus's Mouse Opossum (*Marmosa murina*, Didelphimorphia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. P values in bold represent significant values.

Hylaeamys seuanezi

	Body Weight				
	df	**Sum Sq**	**Mean Sq**	**F value**	**p**
Body Length	1	149.0	149.0	4.616	0.164
Habitat	1	110.4	110.4	3.422	0.205
Body Length : Habitat	1	502.3	502.3	15.563	**0.058**
Residuals	2	64.5	32.3		

Marmosa murina

	Body Weight				
	df	**Sum Sq**	**Mean Sq**	**F value**	**p**
Body Length	1	891.1	891.1	7.203	**0.036**
Habitat	1	105.9	105.9	0.856	0.390
Body Length : Habitat	1	50.6	50.6	0.409	0.545
Residuals	6	742.3	123.7		

Table 12. PERMANOVA results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) and body condition index (BCI) in marsupials (Didelphimorphia) and rodents (Rodentia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; F: F value by permutation and p value: p-values based on more than 9000 permutations (the lowest possible p-value is 0.0001). P values in bold represent significant values.

Didelphimorphia						
			Lead (Pb)			
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	29.4	29.38	0.04764	0.00353	0.844
Habitat	1	202.7	202.74	0.32870	0.02436	0.572
BCI : Habitat	1	71.9	71.93	0.11662	0.00864	0.751
Residuals	13	8018.4	616.80		0.96347	
Total	16	8322.4			1.00000	
			Manganese (Mn)			
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	0.655	0.6553	0.14710	0.01012	0.797
Habitat	1	6.111	61.106	137.174	0.09439	0.239
BCI : Habitat	1	0.062	0.0615	0.01381	0.00095	0.905
Residuals	13	57.910	44.546		0.89454	
Total	16	64.737			1.00000	
			Copper (Cu)			
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	0.9501	0.95012	1.32180	0.07618	0.287
Habitat	1	2.0984	2.09836	2.91922	0.16824	0.112
BCI : Habitat	1	0.0792	0.07920	0.11019	0.00635	0.709
Residuals	13	9.3445	0.71881		0.74923	
Total	16	12.4722			1.00000	
Rodentia						
			Lead (Pb)			
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	8.82	8.821	0.1898	0.01125	0.692
Habitat	1	299.65	299.651	6.4487	0.38228	**0.016**

BCI : Habitat	1	10.70	10.703	0.2303	0.01366	0.654
Residuals	10	464.67	46.467		0.59281	
Total	13	783.85			1.00000	
Manganese (Mn)						
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	5.656	5.6563	0.50670	0.04160	0.753
Habitat	1	15.516	15.5164	1.38998	0.11413	0.261
BCI : Habitat	1	3.151	3.1506	0.28224	0.02317	0.781
Residuals	10	111.631	11.1631		0.82109	
Total	13	135.954			1.00000	
Copper (Cu)						
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	1.1244	1.1244	0.71156	0.06020	0.618
Habitat	1	1.3307	1.3307	0.84210	0.07124	0.485
BCI : Habitat	1	0.4218	0.4218	0.26693	0.02258	0.810
Residuals	10	15.8019	1.5802		0.84598	
Total	13	18.6788			1.00000	

Table 13. PERMANOVA results table for concentration of Lead (Pb), Manganese (Mn) and Copper (Cu) (mg/Kg) and body condition index (BCI) in Atlantic Forest Oryzomys (*Hylaeamys seuanezi*, Rodentia) and Linnaeus's Mouse Opossum (*Marmosa murina*, Didelphimorphia) from the Traditional cacao agroforests and Atlantic Forest fragments in southern Bahia, Brazil. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; F: F value by permutation and p value: p-values based on more than 9000 permutations (the lowest possible p-value is 0.0001). P values in bold represent significant values.

Hylaeamys seuanezi						
			Lead (Pb)			
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	14.402	14.402	18.672	0.07215	0.066
Habitat	1	125.963	125.963	163.308	0.63101	**0.022**
BCI : Habitat	1	57.714	57.714	74.824	0.28912	**0.044**
Residuals	2	1.543	0.771		0.00773	
Total	5	199.622			1.00000	
			Manganese (Mn)			
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	0.059483	0.059483	2.0406	0.24716	0.311
Habitat	1	0.041605	0.041605	1.4273	0.17288	0.444
BCI : Habitat	1	0.081276	0.081276	2.7882	0.33771	0.311
Residuals	2	0.058300	0.029150		0.24225	
Total	5	0.240664			1.00000	
			Copper (Cu)			
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	0.054390	0.054390	17.470	0.26091	0.088
Habitat	1	0.018426	0.018426	5.918	0.08839	0.133
BCI : Habitat	1	0.129417	0.129417	41.570	0.62083	**0.033**
Residuals	2	0.006227	0.003113		0.02987	
Total	5	0.208459			1.00000	
Marmosa murina						
			Lead (Pb)			
	df	**SS**	**MS**	**F**	**R^2**	**p value**

BCI	1	96.3	96.32	0.19028	0.02579	0.822
Habitat	1	28.8	28.77	0.05683	0.00770	0.777
BCI : Habitat	1	573.2	573.16	1.13233	0.15344	0.339
Residuals	6	3037.1	506.18		0.81307	
Total	9	3735.3			1.00000	
Manganese (Mn)						
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	2.965	29.653	0.39823	0.05146	0.645
Habitat	1	8.100	80.996	108.778	0.14057	0.353
BCI : Habitat	1	1.881	18.807	0.25258	0.03264	0.677
Residuals	6	44.676	74.460		0.77534	
Total	9	57.622			100.000	
Copper (Cu)						
	df	**SS**	**MS**	**F**	**R^2**	**p value**
BCI	1	2.7080	2.70798	3.15135	0.24386	0.098
Habitat	1	2.5982	2.59820	3.02360	0.23398	0.132
BCI : Habitat	1	0.6425	0.64252	0.74772	0.05786	0.404
Residuals	6	5.1558	0.85931		0.46430	
Total	9	11.1045			1.00000	