

**AVES MARINHAS COMO SENTINELAS DE SAÚDE AMBIENTAL:
CONTAMINAÇÃO POR METAIS E METALÓIDES E ASSOCIAÇÕES
COM ESTRESSE OXIDATIVO NO RIO DE JANEIRO**

LARA SILVA DE PAULA

UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE – UENF

CAMPOS DOS GOYTACAZES – RJ

MARÇO, 2026

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Orientador: Dr. Salvatore Siciliano

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RESUMO

Metais e metalóides possuem toxicidade e potencial de bioacumulação pertinentes para a saúde ambiental/humana. Aves são importantes sentinelas de contaminação devido à diversidade de hábitos e características biológicas, e monitorar metais em aves marinhas possibilita delimitar um panorama do perfil de contaminação e de qualidade ambiental. O presente trabalho teve como objetivo quantificar concentrações de arsênio (As), cádmio (Cd), chumbo (Pb), ferro (Fe), mercúrio (Hg), rubídio (Rb), selênio (Se), titânio (Ti) e zinco (Zn), além de biomarcadores de estresse oxidativo envolvidos na destoxificação de metais (metalotioneína – MT e glutathiona reduzida – GSH) em tecidos de aves marinhas. As amostras de aves marinhas analisadas foram provenientes de carcaças de cinco espécies, sendo elas *Spheniscus magellanicus*, *Sula leucogaster*, *Fregata magnificens*, *Larus dominicanus* e *Thalasseus maximus* oriundas de coletas na região Norte Fluminense e Baixadas Litorâneas do estado do Rio de Janeiro. Dentre as amostras analisadas, fígado apresentou maiores níveis de Cu, Fe, Zn, Cd, Hg e Ti do que músculo, ademais, de maneira geral Fe, Rb, As, Cd e Hg foram encontrados em maiores concentrações em *S. magellanicus* e *S. leucogaster*. Correlações entre os elementos Se e Hg foram encontradas, bem como razão molar Se:Hg > 1 na maioria das amostras. Além disso, foram encontradas correlações entre biomarcadores e metais (GSH com As, Cd e Ti; MT com Cd, Cu, Fe, Rb e Ti). Dentre os elementos analisados, metais essenciais, As e Pb não indicam riscos de toxicidade, Ti e Rb necessitam de maiores investigações, enquanto baixa capacidade de destoxificação e possíveis efeitos tóxicos podem estar ocorrendo devido a Cd e Hg (especialmente em *S. magellanicus* e *S. leucogaster*).

Palavras-chave: metais; metalóides; aves marinhas; bioindicadores

ABSTRACT

Metals and metalloids are toxic and have bioaccumulation potential, which are pertinent for environmental/human health. Avian species are important sentinels of contamination due to their diversity of habits and biological characteristics, and monitoring metals in seabirds enables an assessment of environmental contamination and quality. The objective of this study was to quantify concentrations of arsenic (As), cadmium (Cd), lead (Pb), iron (Fe), mercury (Hg), rubidium (Rb), selenium (Se), titanium (Ti), and zinc (Zn), in addition to biomarkers of oxidative stress and detoxification (metallothionein – MT, and reduced glutathione – GSH) in tissues of seabirds. Seabirds samples analyzed here are originated from carcass of five species, namely *Spheniscus magellanicus*, *Sula leucogaster*, *Fregata magnificens*, *Larus dominicanus*, and *Thalasseus maximus* from sampling in the Norte Fluminense and Baixadas Litorâneas regions of Rio de Janeiro state. Among the analyzed samples, liver presented higher levels of Cu, Fe, Zn, Cd, Hg and Ti than muscle, additionally, Fe, Rb, As, Cd, and Hg are found in higher concentrations in *S. magellanicus* and *S. leucogaster*. Correlation between Se and Hg were found, as well as molar ratio $\text{Se:Hg} > 1$ in most of the samples. Additionally, correlations between biomarker and metals were found (GSH with As, Cd, and Ti; Mt with Cd, Cu, Fe, Rb, and Ti). Among the investigated elements, essential metals, As and Pb do not present toxicity risk, more investigations are needed for Ti and Rb, additionally, low detoxification capacity and possible toxic effects could be happening due to Cd and Hg (especially in *S. magellanicus* and *S. leucogaster*).

Keywords: metals; metalloids; seabirds; bioindicators

INTRODUÇÃO GERAL

Metais e metalóides como contaminantes ambientais

Ambientes marinhos e costeiros são constantemente expostos a diversas fontes de contaminantes provenientes de atividades antrópicas. Estes poluentes incluem metais e metalóides, que embora sejam mobilizados através de processos biogeoquímicos naturais, são emitidos, em grande parte, por atividades antrópicas (Vizuite et al., 2018; Wallig et al., 2018; Kendall et al., 2010; He et al., 2005). Dentre as fontes antropogênicas de emissão de metais e metalóides, destacam-se as atividades industriais (como fabricação de baterias, eletrônicos, pigmentos e plástico), a mineração e metalurgia, a queima de combustíveis fósseis, as atividades agrícolas (uso de pesticidas e fertilizantes) e os efluentes (tanto industriais como urbanos) (Bahamonde et al., 2023; Rodrigues et al., 2022a; Vizuite et al., 2018; Mirlean e Roisenberg, 2006).

No ambiente, metais e metalóides podem ser acumulados por organismos através de diferentes processos. A bioconcentração e bioacumulação ocorrem quando esses elementos são absorvidos diretamente do ambiente através da via respiratória ou por meio da ingestão de água e alimento (Burger e Gochfeld, 2004; Gray, 2002). Além disso, as concentrações de alguns metais e metalóides, como mercúrio, zinco e chumbo, podem aumentar ao longo dos níveis tróficos, em um processo denominado biomagnificação (Wang et al., 2021; Sun et al., 2020; Vizuite et al., 2018; Burger e Gochfeld, 2004; Gray, 2002).

Elementos não-essenciais, como arsênio (As), cádmio (Cd), chumbo (Pb) e mercúrio (Hg), não possuem função biológica e podem exercer toxicidade mesmo em baixas concentrações, e por essa razão, são considerados importantes agentes de contaminação ambiental e de riscos à saúde pública (Lima et al., 2023; Matias et al., 2022; Rodrigues et al., 2022a; Celik et al., 2021; Vizuite et al., 2018; Carravieri et al., 2014; Burger et al., 2007). Uma grande parcela da deposição de As, Cd, e Pb em diversos ecossistemas resulta de atividades antrópicas relacionadas à mineração e metalurgia, atividades industriais (como a fabricação de eletrônicos, baterias, pigmentos, plástico e fertilizantes), queima de combustíveis fósseis, e ao uso em defensivos agrícolas, enquanto a emissão de Hg está associada, além de atividades industriais e metalurgia, às atividades garimpeiras, queima de carvão

mineral, e efluentes urbanos e industriais (Bahamonde et al., 2023; Rodrigues et al., 2022a; Vizuite et al., 2018; Mirlean e Roisenberg, 2006).

Metais como cobre (Cu), ferro (Fe) e zinco (Zn) são essenciais para organismos vivos (Celik et al., 2021), atuando na manutenção da homeostase através de processos de síntese proteica, regulação enzimática, metabolismo de carboidratos e regulação do sistema antioxidante (Hauser-Davis et al., 2023a; Lima et al., 2023; Rodrigues et al., 2022a; Wallig et al., 2018). Contudo, concentrações elevadas desses metais podem gerar efeitos tóxicos como lesões hepáticas, distúrbios hormonais e disfunção imune, sendo então considerados potenciais contaminantes ambientais (Bahamonde et al., 2023; Lima et al., 2023; Rodrigues et al., 2022a; Celik et al., 2021; Ebert et al., 2020; Sobhakumari et al., 2018; Vizuite et al., 2018; Wallig et al., 2018). Embora Cu, Fe e Zn possuam efluentes industriais e urbanos, e a queima de combustível fóssil como fontes antropogênicas em comum, a emissão de Cu pode ainda estar associada à indústria metalúrgica, ao uso em tintas anti-incrustantes para navios, fertilizantes e a produtos médicos/farmacêuticos como biocidas, enquanto o Fe também pode ser emitido para o ambiente através da produção de cimento e de vidro (Rathod et al., 2024; Rodrigues et al., 2022a; Celik et al., 2021; Chen et al., 2021; Bighiu et al., 2017; Lee et al., 2010; Vaalgamaa e Conley, 2008).

Outros metais, como rubídio (Rb) e titânio (Ti), contam com poucos estudos sobre seus mecanismos de toxicidade e comportamento ao longo de cadeias tróficas, sendo estes considerados contaminantes de preocupação emergente (Bahamonde et al., 2023; Rodrigues et al., 2022b; Hauser-Davis et al., 2020; Wallig et al., 2018). Embora exista pouca informação sobre o potencial de bioacumulação de Rb ao longo dos níveis tróficos, concentrações em organismos, limites tóxicos e mecanismos de toxicidade (Bahamonde et al., 2023; Haas e Kočvara, 2023; Hauser-Davis et al., 2023b), Campbell et al. (2005) e Souza et al. (2021) sugerem que esse elemento possui capacidade de bioacumular ao longo de níveis tróficos em ecossistemas marinhos e estuarinos. Além disso, o Ti e as nanopartículas de dióxido de titânio (TiO₂-NP) possuem potencial de gerar efeitos tóxicos e bioacumulação em organismos aquáticos (Chen et al., 2011). Dentre as atividades antrópicas que emitem Rb pode-se destacar queima de carvão, uso em aparelhos médicos e

dispositivos eletrônicos (Hauser-Davis et al., 2023b; Campbell et al., 2005). Por outro lado, o Ti é amplamente utilizado na forma de TiO₂-NP na indústria alimentícia e farmacêutica, fabricação de pigmentos, protetor solar e cosméticos, além do uso como catalisadores para purificação de ar e água (Nam et al., 2014; Weir et al., 2012; Nowack e Bucheli, 2007).

Efeitos fisiológicos de metais e metalóides em aves e outros organismos vivos

Os organismos vivos são expostos a inúmeros estressores no decorrer da vida, podendo desencadear efeitos fisiológicos, comportamentais e/ou reprodutivos, diretos ou indiretos, que comprometem a estabilidade das populações ao longo do tempo (Celik et al., 2021; Petitjean et al., 2020; Ding et al., 2019; Grunst et al., 2018; Kendall et al., 2010; Eeva et al., 2005). Muitos metais exercem toxicidade por meio da indução de estresse oxidativo, que pode ocorrer de duas formas principais: (I) pela geração direta de espécies reativas de oxigênio (EROs) ou (II) pela redução da eficiência do sistema antioxidante (Koivula e Eeva, 2010). Quando o estresse oxidativo gerado pelas EROs não é adequadamente controlado pelo sistema antioxidante, podem ocorrer danos significativos ao DNA, proteínas e membrana celular (Koivula e Eeva, 2010). Nesse contexto, os antioxidantes possuem um papel fundamental ao inibir a formação de radicais livres e ao eliminá-los, reduzindo a oxidação e minimizando os danos potenciais (Koivula e Eeva, 2010).

Contudo, além dos limites considerados tóxicos, que variam para cada espécie, a toxicidade de um metal é influenciada por diversos fatores. Interações químicas com outros elementos, forma química, mecanismos de absorção, bioacumulação, e posterior vias de destoxificação e excreção, são determinantes para sua ação, e portanto, a toxicidade não pode ser avaliada exclusivamente através das concentrações totais de metais e metalóides no organismo/tecido (Hauser-Davis et al., 2023a; Kojadinovic et al., 2007). Para uma melhor compreensão dos efeitos, é necessário associar os níveis totais a outros fatores, como biodisponibilidade em frações intracelulares, níveis de metalotioneína (MT) e resposta antioxidante (e.g., glutathione reduzida - GSH).

Entre os metalóides destaca-se o arsênio que pode apresentar diferentes níveis de toxicidade a depender de sua forma química/especiação (Zhuang et al.,

2023). Dentre as formas químicas do arsênio estão as formas inorgânicas arsenito (trivalente – As(III) - AsO_3^{3-}) e arsenato (pentavalente – As(V) - AsO_4^{3-}), sendo essas geralmente mais tóxicas, e formas orgânicas e metiladas deste metaloide com menor toxicidade (Zhuang et al., 2023; Rodrigues et al., 2022a; Furtado et al., 2019; Vizuite et al., 2018; Kumari et al., 2017; Barra et al., 2000). Arsênio, principalmente quando em formas inorgânicas trivalentes (arsenito, com elevada toxicidade), possui grande afinidade por grupamento tiol (i.e. sulfidril - SH), podendo se ligar a cisteína, peptídeos e proteínas nos organismos (Shen et al., 2013). A toxicidade do As é dada pela sua interferência no sistema antioxidante, reduzindo a atividade de enzimas como Superóxido Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) e diminuindo os níveis de GSH, o que culmina na produção de EROs e Espécies Reativas de Nitrogênio (ERNs), que causam danos às membranas lipídicas e ao DNA (Vizuite et al., 2018; Wallig et al., 2018). Os efeitos nocivos aos animais incluem toxicidade renal, danos hepáticos, efeitos neurológicos devido ao potencial de ultrapassar a barreira hematoencefálica, gastroenterites, colites, e supressão do sistema hematopoiético através de danos a membrana de eritrócitos, hemólise, liberação do grupamento heme e pancitopenia (Vizuite et al., 2018; Wallig et al., 2018). Além disso, em aves, gera prejuízos reprodutivos como diminuição da taxa de postura e da espessura de cascas de ovos (Celik et al., 2021; Vizuite et al., 2018).

De forma semelhante ao As, o cádmio (Cd) é um metal amplamente estudado devido aos seus efeitos tóxicos, que também estão relacionados ao estresse oxidativo. Contudo, os mecanismos de ação e os órgãos-alvo do Cd apresentam algumas diferenças. A toxicidade do Cd está relacionada à indução de estresse oxidativo, através da diminuição da atividade antioxidante e produção de radicais livres (Li et al., 2021; Zhu et al., 2020). Rins e fígado, principais órgãos de deposição de Cd (Zhu et al., 2020; Burger, 2008), apresentam maior susceptibilidade ao desenvolvimento de lesões, como fibrose hepática, infiltrado de células inflamatórias na região periportal, hiperplasia de ducto biliar, e apoptose de células renais durante a hidrólise de compostos de Cd-MT (Zhu et al., 2020; Wallig et al., 2018; Burger, 2008). Além disso, o Cd gera distúrbios comportamentais e reprodutivos em aves, causando lesões testiculares, diminuição da espessura de cascas de ovos (Celik et al., 2021; Burger, 2008) e patologias cardíacas, como congestão, desarranjo e

ruptura de fibras do miocárdio, hiperplasia, lise de membrana e vacuolização de mitocôndria (Li et al., 2021).

O Pb possui toxicidade nos sistemas renal, hepático, hematopoiético, reprodutivo e nervoso. Como no organismo o Pb é carregado por eritrócitos, pode causar anemia, tanto por hemólise, quanto por diminuição da produção medular de hemácias (Celik et al., 2021; Sobhakumari et al., 2018; Wallig et al., 2018). A intoxicação por Pb em aves pode apresentar sinais clínicos inespecíficos (Sobhakumari et al., 2018), como diminuição de massa, apetite e ingestão de alimento (Al-Mansour et al., 2009). Além disso, este metal pode ocasionar problemas reprodutivos em aves, tais como impactos negativos no crescimento e sobrevivência de filhotes, redução de plasma em ovos e diminuição das taxas de incubação (Celik et al., 2021). A toxicidade hepática do Pb, já foi documentada através de achados de necrópsia, como aparência esverdeada no fígado e aumento da vesícula biliar com estase da bile (Sobhakumari et al., 2018), assim como o dano hepático progressivo em *Coturnix coturnix* (Codorniz-comum) expostas a doses sub tóxicas deste metal (Al-Mansour et al., 2009). Além disso, o Pb também é um precursor de lesão celular em rins, apresentando quadros de necrose com corpos de inclusão intranucleares em células epiteliais do túbulo renal proximal (Sobhakumari et al., 2018; Wallig et al., 2018).

Em se tratando de Hg, pode ser encontrado no ambiente nas formas elementar (Hg^0), inorgânica (Hg^{2+}) ou orgânica (e.g. CH_3Hg^+ – metilmercúrio – MeHg) (Rodrigues et al., 2022a; Vizuete et al., 2018). O MeHg é a principal forma de contaminação por mercúrio orgânico em organismos vivos, e possui elevada capacidade de bioacumulação e biomagnificação ao decorrer da teia alimentar (Rodrigues et al., 2022a; Sobhakumari et al., 2018). Além disso, este composto orgânico é citotóxico e lipofílico, podendo causar alterações hormonais, reprodutivas, comportamentais e neurológicas, com capacidade de atravessar a barreira hematoencefálica, prejudicar a neurotransmissão, e acarretar desmielinização e necrose de nervos (Sobhakumari et al., 2018). No contexto reprodutivo de aves, a exposição ao Hg contribui com a redução de performance, levando à diminuição da produção, fertilização e espessura de cascas de ovos, além de ter efeitos embriotóxicos que resultam na mortalidade de embriões (Celik et al., 2021; Ma et al.,

2018; Sobhakumari et al., 2018; Wallig et al., 2018). Ademais, pode ocorrer transferência materna tanto de Hg como MeHg para prole (Ackerman et al., 2016). O Hg interfere na fosforilação oxidativa nas mitocôndrias, resultando em hipóxia e apoptose de células do epitélio tubular renal, além disso, processos de peroxidação lipídica também podem ser considerados como mecanismos de toxicidade (Wallig et al., 2018), culminando em danos como necrose renal (Sobhakumari et al., 2018) e esteatose hepática (Ma et al., 2018).

Ao contrário dos elementos não-essenciais abordados anteriormente nesta subseção, os metais essenciais possuem diversas funções importantes no organismo. Dentre as funções do Cu, destaca-se seu papel como componente e cofator de diversas enzimas (Jomova et al., 2022). Já o Fe integra o grupamento heme de hemoglobinas (Casa-Resino et al., 2014), enquanto o Zn está envolvido em processos neuronais, imunes, reprodutivos, enzimáticos, expressão gênica, sistema antioxidante, dentre outros (Jomova et al., 2022). No entanto, em elevadas concentrações, esses metais essenciais podem gerar efeitos tóxicos.

Cobre, Fe e Zn possuem potencial de mediar a formação de EROs, causarem danos mitocondriais e danos oxidativos em membranas (Jomova et al., 2022; Celik et al., 2021; Sobhakumari et al., 2018). A produção de EROs pelo Fe ocorre principalmente quando esse metal se encontra livre, e outros metais, como Pb e Cd, facilitam a liberação de Fe das moléculas de ferritina no organismo, facilitando a formação de EROs (Koivula e Eeva, 2010). Além disso, as concentrações elevadas de Cu podem causar danos diversos em aves, como redução na produção de ovos, perda de peso, problemas no crescimento de penas e falhas na função renal (Celik et al., 2021; Koivula e Eeva, 2010).

Além dos efeitos diretamente causados por metais não-essenciais, metalóides (As), e metais essenciais, outros contaminantes podem interagir com esses elementos, agravando seus efeitos tóxicos. Os efeitos tóxicos de Rb e Ti em aves são pouco conhecidos. Segundo Song et al. (2016), TiO_2 -NP levam à indução de estresse oxidativo em variados tecidos, assim como danos em mitocôndrias, desencadeamento de resposta inflamatória, apoptose e efeitos genotóxicos, sendo esses os possíveis mecanismos de toxicidade. Em culturas celulares e outros organismos (como ratos, *Danio rerio*, *Trachinotus carolinus* e *Cyprinus carpio*), TiO_2 -

NP já foram descritas como causadoras de citotoxicidade e genotoxicidade, hemólise e alteração morfológica em macrófagos, além da formação de EROs e diminuição de GSH (Chen et al., 2019; Allegri et al., 2016; Vignardi et al., 2015). Além disso, devido às características físico químicas de TiO_2 -NP, como sua elevada superfície de contato, essas partículas são capazes de adsorver contaminantes, incluindo metais como Pb, Cd, Cu, Zn e o metalóide As (Engates e Shipley, 2011; Sun et al., 2007; Zhang et al., 2007), atuando como carreadoras de metais, e portanto, facilitando a bioacumulação desses elementos nos organismos (Yang et al., 2014; Sun et al., 2007; Zhang et al., 2007). Desta forma, além de avaliar os possíveis efeitos que as TiO_2 -NP causam diretamente a organismos, é necessário levar em consideração o potencial de alterar a toxicidade de outros contaminantes (Zhang et al., 2007).

Por outro lado, outro elemento que deve ser destacado tanto por seu potencial toxicidade quanto por sua capacidade de destoxificação, é o selênio (Se). O Se é um não-metal que também pode ser considerado indutor de estresse oxidativo, levando a quadros de toxicidade que incluem efeitos danosos no fígado, sistema nervoso e hematopoiético, entretanto, este elemento é essencial para manutenção da homeostase, e participa de diversos processos no organismo, incluindo a composição de selenoproteínas e selenoenzimas, regulação enzimática, auxílio ao sistema imune e sistema antioxidante (Burger et al., 2023; Burger et al., 2019; Pilarczyk et al., 2019). Além disso, o Se pode contribuir para a destoxificação de metais através da indução da síntese de proteínas capazes de se ligar a metais, ou então por meio da sua ligação direta com metais (incluindo Cd, Pb, Hg e MeHg), formando compostos selenitos/selenetos (Pilarczyk et al., 2019; Nam et al., 2005). Kehrig et al. (2015) apresentaram a relação entre níveis de Se e concentrações de Hg, Cd e Pb no fígado de *Spheniscus magellanicus*, sugerindo o processo de destoxificação associado ao Se.

Mesmo o selênio sendo reportado como atuante na destoxificação de diferentes metais, como o Cd e o Pb, um destaque maior é dado para sua relação com Hg. Cabañero et al. (2006) reportaram aumento de 145-280% da desmetilação de MeHg em fígado e rins de galinhas quando expostas a dietas contendo MeHg em conjunto com Se. Um estudo com aves marinhas (El Hanafi et al., 2022) sugere que

o Se, na forma de selenoneína, estaria diretamente envolvido no processo de desmetilação de MeHg e na posterior formação de complexos HgSe, evidenciado pela correlação entre o aumento de Hg acumulado no fígado e a diminuição dos níveis de selenoneína, possivelmente devido ao uso desse aminoácido no processo de desmetilação, que resultaria na degradação tanto do MeHg quanto da selenoneína. Como tanto o Se quanto o Hg possuem potencial toxicidade e elevada afinidade entre si, a literatura sugere a existência de um mecanismo de acumulação conjunta entre esses elementos, que seria capaz de atenuar ambas as toxicidades (Burger et al., 2019; Lucia et al., 2012). Diversos estudos experimentais e com mamíferos marinhos reportam o potencial efeito protetor do Se contra o Hg, indicando a possibilidade de ocorrência deste efeito também em aves marinhas (González-Solís et al., 2002; Hutton, 1981). Portanto, a ligação entre esses elementos formaria seleneto de mercúrio (HgSe), acumulando esses metais de uma forma não tóxica (Lucia et al., 2012).

A razão molar Se:Hg encontrada em mamíferos marinhos é de 1:1, enquanto em outros organismos marinhos, como moluscos, crustáceos e aves, a quantidade de Se é comumente superior a de Hg (Dietz et al., 2000). Estudo de Burger et al. (2019) encontrou uma razão molar entre Se:Hg > 7500:1 em *Calidris pusilla* (maçarico-rasteirinho), enquanto Lucia et al. (2012) observaram a razão molar Se:Hg sempre maior que 1 em fígado de *Limosa limosa* (maçarico-de-bico-direito), sugerindo que o selênio possui efeito protetor contra Hg em níveis onde a razão molar Se:Hg seja > 1 em aves (Hauser-Davis et al., 2023a; Burger et al., 2019; Lucia et al., 2012). Ainda assim, são escassos estudos experimentais que comprovem o efeito protetor e qual a razão considerada protetora Se:Hg (Burger et al., 2023), principalmente levando em consideração aves marinhas pertencentes à fauna silvestre como objetos de estudo.

Alguns tripeptídeos e proteínas, por exemplo a GSH, e metaloproteínas, como a MT são fundamentais nos processos de homeostase e destoxificação de metais, sendo capazes de sequestrá-los e carregá-los, além de integrarem o sistema antioxidante (Monteiro et al., 2019).

A GSH é um tripeptídeo que possui grupamento tiol (i.e., sulfidril – SH) e está envolvido no sistema antioxidante. Essa molécula atua na primeira linha de

defesa contra o estresse oxidativo causado por metais, agindo tanto diretamente sobre o estresse gerado ou então através da formação de complexos com metais levando a destoxificação dos mesmos (Monteiro et al., 2019; Kehrig et al., 2016; Lavradas et al., 2014; Koivula e Eeva, 2010; Forman et al., 2009; Wang et al., 2008; Canesi et al., 1999). Alterações nos níveis de GSH podem indicar estresse oxidativo, inclusive induzido por metais. Os níveis de GSH podem ser afetados de diferentes formas a depender da espécie e tecido analisado, assim como do metal em questão e de sua concentração (Monteiro et al., 2019; Kehrig et al., 2016; Srikanth et al., 2013). Estudos demonstram tanto o aumento de GSH em aves de locais contaminados por metais, quanto sua diminuição (Koivula e Eeva, 2010).

As MT são proteínas de baixo peso molecular e ricas em cisteína que possuem capacidade de se ligar a metais, tendo sua síntese induzida pela presença dos mesmos no organismo (Wallig et al., 2018; Kehrig et al., 2015; Nam et al., 2005; Andrews et al., 1996). Metais como Cu, Cd e Zn em aves foram reportados por Nam et al. (2005) estando ligados a MT. Essa ligação de metais com MT ocorre através de seu grupamento tiol (i.e., sulfidril - SH), em um processo de quelação e formação de ligações metal-tiolato (Koivula e Eeva, 2010; Andrews et al., 1996). Essa proteína se liga tanto a metais essenciais como Zn e Cu (sendo responsáveis pela regulação da homeostase), quanto não-essenciais como Cd, Hg e Pb (atuando como reservatório e mecanismo de destoxificação) (Monteiro et al., 2019; Wallig et al., 2018; Kehrig et al., 2015; Koivula e Eeva, 2010; Kojadinovic et al., 2007; Nam et al., 2005; Andrews et al., 1996).

Aves marinhas como sentinelas de contaminação por metais e metalóides

Por conta da ampla distribuição geográfica e do elevado nível trófico (predadoras e/ou necrófagas) de aves marinhas, estes animais são frequentemente expostos a diversos contaminantes, sendo passíveis de bioacumulação e biomagnificação, destacando-os como importantes organismos bioindicadores e sentinelas para estudos de contaminação ambiental (Nunes et al., 2023; Celik et al., 2021; Vizuite et al., 2018; Burger e Gochfeld, 2004). Neste contexto, a escolha da matriz a ser analisada varia de acordo com o tipo de contaminante e a finalidade do estudo, sendo as mais comumente utilizadas para biomonitoramento de aves

marinhas ovos, penas, fígado, rins e sangue (Bahamonde et al., 2023; Lehel et al., 2023; Mukhtar et al., 2020; Vizuite et al., 2018; Burger e Gochfeld, 2004; Burger e Gochfeld, 2000). Existem diferenças com relação aos níveis de metais e metalóides depositados em cada tecido devido a características dos elementos e da fisiologia do organismo/tecido (Khwankitritikul et al., 2024; Lehel et al., 2023; Mukhtar et al., 2020; Vizuite et al., 2018). Órgãos como fígado e rins tendem a acumular maiores concentrações de metais e metalóides e indicam exposição crônica, tendo em vista que esses órgãos são responsáveis por processos de destoxificação (Khwankitritikul et al., 2024; Lehel et al., 2023; Rodríguez-Álvarez et al., 2022; Mukhtar et al., 2020; Janaydeh et al., 2016), enquanto músculo representa apenas um tecido alvo da deposição destes contaminantes (Rodríguez-Álvarez et al., 2022; Janaydeh et al., 2016).

A avaliação das concentrações de metais e metalóides se faz uma estratégia importante de monitoramento desses contaminantes e de seus impactos nas populações de aves marinhas (Burger et al., 2023; Vizuite et al., 2018; Kendall et al., 2010; Burger et al., 2004). Além de fornecer um perfil da saúde ambiental e da saúde de espécies silvestres, o monitoramento contribui com a análise de risco e previsão de problemas futuros, viabilizando estratégias de mitigação (Couto e Ribeiro, 2022; Calado et al., 2021; Gyamfi et al., 2021; Marques et al., 2021; Vizuite et al., 2018; Ferreira, 2011; Kendall et al., 2010; Peakall e Burger, 2003).

De maneira geral, são escassos estudos no Brasil que avaliem níveis de metais e metalóides em aves marinhas, principalmente levando em conta outros fatores além da concentração total destes contaminantes em um tecido específico. A fim de compreender como esses contaminantes se comportam no organismo de aves marinhas, se torna necessária a pesquisa de concentrações em diferentes tecidos no mesmo indivíduo, bem como a avaliação de mais de uma espécie. Além disso, a associação de níveis totais de metais e metalóides com biomarcadores como MT e GSH é uma ferramenta importante para entender a sua biodisponibilidade e possíveis efeitos tóxicos.

OBJETIVOS

Objetivo Geral

Investigar a presença e concentrações de metais e metalóides e de biomarcadores em amostras biológicas de cinco espécies de aves marinhas: pinguim-de-magalhães (*Spheniscus magellanicus*), atobá-marrom (*Sula leucogaster*), gaivotão (*Larus dominicanus*), fragata (*Fregata magnificens*) e trinta-réis-real (*Thalasseus maximus*).

Objetivos Específicos

- Determinar as concentrações de As, Cd, Cu, Fe, Hg, Pb, Rb, Se, Ti e Zn em amostras de músculo e fígado *S. magellanicus*, *S. leucogaster*, *F. magnificens*, *L. dominicanus* e *T. maximus*, de carcaças oriundas do Norte Fluminense e Baixadas Litorâneas do estado do Rio de Janeiro;
- Dosar a proteína metalotioneína (MT) e o tripeptídeo glutathiona (GSH) em amostras de músculo e fígado de *S. magellanicus*, *S. leucogaster*, *F. magnificens*, *L. dominicanus* e *T. maximus*;
- Correlacionar os níveis de MT, GSH e Se com concentrações de metais e metalóides;
- Comparar as concentrações de contaminantes entre as espécies e tecidos.

HIPÓTESES

Hipótese I: É esperado que existam relações variáveis (positivas ou negativas) entre os níveis dos biomarcadores (MT e GSH) e as concentrações dos diferentes metais e metaloides devido a processos de controle de estresse oxidativo e destoxificação.

Hipótese II: A razão Se:Hg será compatível com casos de exposição a Hg com potencial destoxificação (razão Se:Hg > 1), devido a elevada exposição deste metal em aves marinhas e as mesmas apresentarem maior tolerância a quadros de intoxicação por Hg.

Hipótese III: Por apresentarem diferentes hábitos alimentares e de forrageio, as espécies de aves marinhas *S. magellanicus*, *S. leucogaster*, *F. magnificens*, *L. dominicanus* e *T. maximus* apresentam variações interespecíficas no acúmulo de metais e metaloides. É esperado que *S. leucogaster* apresente elevados níveis de Hg e *S. magellanicus* de Cd, por conta de seus hábitos alimentares, enquanto *F. magnificens* e *L. dominicanus* apresentem elevados níveis de metais/metaloides não-essenciais no geral (As, Cd, Hg e Pb), sendo maiores em *L. dominicanus* por possuírem maior contato com ambientes antropizados.

CAPÍTULO I

Artigo submetido ao periódico “Ecotoxicology and Environmental Safety” (Fator de Impacto 2024: 6.1)

METAL AND METALLOID CONTAMINATION AND ASSOCIATIONS WITH OXIDATIVE STRESS BIOMARKERS IN FIVE SEABIRD SPECIES SAMPLED OFF THE SOUTHEASTERN BRAZILIAN COAST

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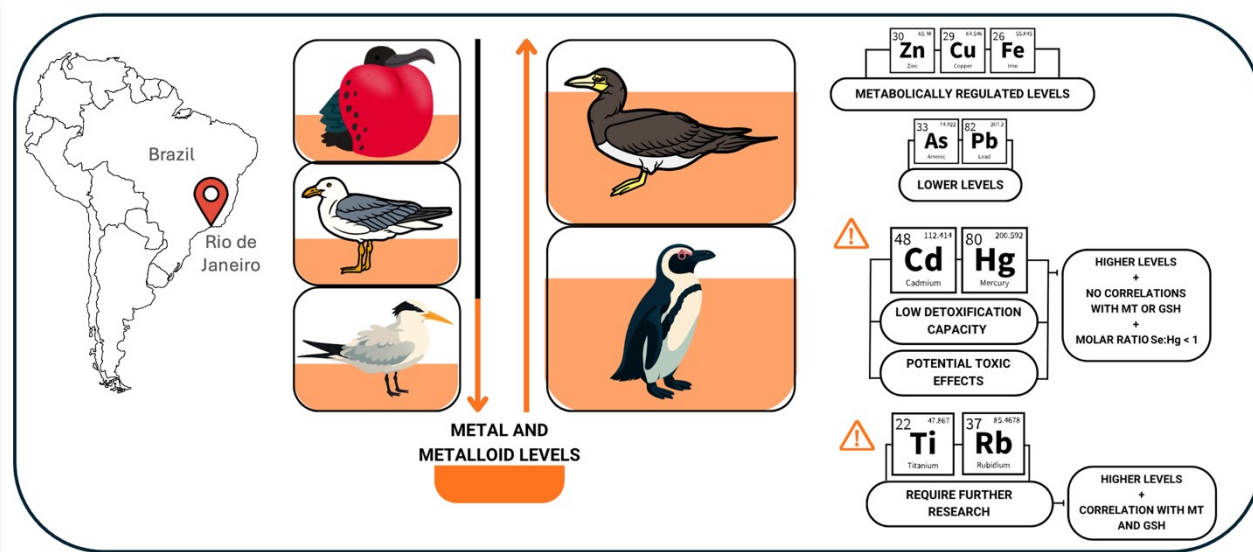
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Graphical abstract



Abstract

Assessing contaminant burdens in seabirds provides valuable insights into environmental quality and species-specific exposure patterns. This study quantified total As, Cd, Cu, Fe, Hg, Pb, Rb, Se, Ti, and Zn, along with two oxidative stress biomarkers involved in metal detoxification, metallothionein (MT) and reduced glutathione (GSH), in the liver and muscle of five seabird species (*Spheniscus magellanicus*, *Sula leucogaster*, *Fregata magnificens*, *Larus dominicanus*, and *Thalasseus maximus*) from southeastern Brazil. Metal concentrations were determined via ICP-MS, and biomarkers via UV-Vis spectrophotometry in 56 muscle and 36 liver samples. Liver generally presented higher levels of Cd, Cu, Fe, Hg, Se, Ti, and Zn. Overall, As, Cd, Fe, Hg, Rb, and Se were higher in *S. magellanicus* and *S. leucogaster*. Although MT and GSH did not differ among species, both were significantly higher in liver than muscle. Significant correlations were observed between As, Cd, and Ti with GSH, between Cd, Cu, Fe, Rb, and between Ti and MT. Similar levels of Cu, Fe, and Zn across species, values below toxicity thresholds, and correlations with MT suggest metabolic regulation of these metals. Mercury detoxification is primarily associated with Se rather than MT or GSH. Low Pb levels and interspecific differences in As highlight the need for further research, including As speciation analyses and the investigation of Ti sources and biomagnification. Overall, this study reveals important interspecific variation and

antioxidant responses in seabirds, emphasizing key knowledge gaps for future assessments.

Keywords: Antioxidant system; Metallothionein; Metals; Reduced glutathione; Sentinel species.

Highlights

- Metal and metalloids were determined in seabirds from Brazil by ICP-MS.
- Higher Cd, Cu, Fe, Hg, Se, Ti, and Zn were detected in liver compared to muscle.
- *S. magellanicus* and *S. leucogaster* presented generally higher As, Cd, Fe, Hg, Rb, and Se.
- Analyzed seabirds typically presented molar ratio Se:Hg > 1 in both tissues.
- As, Cd, and Ti and GSH and MT and Cd, Cu, Fe, Rb, and Ti were positively correlated.

Introduction

Marine environments are continuously exposed to a wide array of contaminants, including metals and metalloids, which may originate from natural biogeochemical processes but are now predominantly linked to anthropogenic activities (Celik et al., 2021; Vizuite et al., 2018). In rapidly urbanizing coastal regions, these inputs tend to accumulate in sediments and biota, amplifying exposure risks for upper-trophic organisms.

Metals and metalloids accumulate in organisms through bioconcentration and bioaccumulation, primarily via the respiratory and digestive pathways, and may biomagnify along food webs (Sun et al., 2020; Vizuite et al., 2018). Such accumulation can result in physiological, behavioral, and reproductive impairments, ultimately threatening population stability (Celik et al., 2021). Several factors influence the bioaccumulation of metals and metalloids in animals, such as age and sex, as well as environmental aspects (*i.e.*, background metal levels, contamination sources, and physicochemical characteristics) (Lemesle et al., 2024; Correia et al., 2023; Anderson et al., 2010). However, diet, trophic level, foraging area, and types of

prey are typically key factors regarding distinct accumulation patterns between animal species (Lemesle et al., 2024; Correia et al., 2023; Padilha et al., 2021; Moura et al., 2018; Becker et al., 2016).

Different metals and metalloids have particularities regarding their deposition rates in organs and tissues. The liver is responsible for various metabolic processes and xenobiotic detoxification, usually accumulating higher levels of contaminants compared to muscle (Bahamonde et al., 2023). Metal and metalloid bioaccumulation in muscle usually takes place following high or chronic exposure scenarios, resulting in overloaded liver capacity and remobilization to other tissues with lower metabolic rates (Aazami et al., 2011). However, studies on metal and metalloid accumulation patterns in different tissues investigating species-specific differences are still scarce for seabirds.

A common toxicity mechanism associated with metals and metalloids is the induction of oxidative stress, marked by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, causing lipid peroxidation, protein oxidation, and DNA damage (Koivula and Eeva, 2010). To deal with this stress, metal-exposed organisms activate physiological responses, particularly detoxification pathways involving the metal-binding protein metallothionein (MT) and antioxidant defense mechanisms mediated by reduced glutathione (GSH) (Monteiro et al., 2019; Kehrig et al., 2016). Reduced glutathione can neutralize ROS and free radicals (Byeon et al., 2021; Srikanth et al., 2013; Koivula and Eeva, 2010), serve as substrate for antioxidant enzymes (Srikanth et al., 2013), and bind metals through sulfhydryl groups (Srikanth et al., 2013; Koivula and Eeva, 2010). Following metal exposure, decrease GSH levels can occur due to the use of glutathione stocks in antioxidant activities, resulting in decreased GSH and increased Oxidized glutathione (GSSG), with a balance concerning GSH synthesis and regeneration required to protect against oxidative stress (Srikanth et al., 2013). Metallothioneins are thermostable, low weight and cysteine-rich proteins with the potential to bind several metals through sulfhydryl group (Koivula and Eeva, 2010), making them unavailable to interact with cellular components (Jakimska et al., 2011; Koivula and Eeva, 2010). Metallothioneins are important both in the regulation of essential metals levels and the detoxification and excretion of non-essential metals (Jakimska et al., 2011;

Koivula and Eeva, 2010), and exposure to different metals lead to different MT induction of synthesis rates (Koivula and Eeva, 2010; Nam et al., 2008; Scheuhammer, 1987).

Seabirds are recognized as effective marine contamination biomonitors due to their wide geographic distribution, long life spans, and high trophic levels (Celik et al., 2021; Vizuite et al., 2018). In this sense, monitoring metal and metalloid concentrations in seabirds provides valuable information on exposure patterns and supports environmental risk assessment and mitigation efforts. A variety of biological seabird matrices, such as eggs, feathers, blood, kidneys, muscle, and liver, among others, are commonly employed in ecotoxicological studies (Bahamonde et al., 2023; Lehel et al., 2023). However, although some toxicological thresholds have been proposed for some avian taxa (Ackerman et al., 2023; Varagiya et al., 2022; Sobhakumari et al., 2018), species-specific limits for wild seabirds remain scarce. This gap is particularly relevant for the southwestern Atlantic, a region experiencing increasing anthropogenic pressures but limited long-term contaminant monitoring.

Despite the importance of integrating biochemical responses into ecotoxicological assessments, studies combining metal quantification with oxidative stress biomarkers in seabirds are still limited. In this context, the present study aimed to characterize the concentrations of several metals and metalloids in the liver and muscle of five seabird species from southeastern Brazil and evaluate potential associations with Se, MT, and GSH. This integrative approach seeks to improve understanding of species-specific detoxification strategies and identify potential early-warning indicators of contaminant exposure in coastal ecosystems.

Material and methods

Study area and seabird species

Samples were obtained from wrecked seabird carcasses (decomposition code 2-3; Fresh and Moderate Decomposition) collected through Beach Monitoring Project and research activities conducted by GEMM-Lagos (Study Group of Marine Mammals from Eastern Rio de Janeiro state coast) between 2007 and 2012. Samples were obtained from the municipalities of Araruama, Arraial do Cabo, Saquarema, Cabo Frio, Armação

dos Búzios, São Francisco de Itabapoana, and Quissamã in the state of Rio de Janeiro, Southeastern Brazil (Figure 1).

Muscle (n = 56) and liver (n = 36) samples were collected from five seabird species, namely Magellanic Penguins (*Spheniscus magellanicus*), Brown Boobies (*Sula leucogaster*), Magnificent Frigatebirds (*Fregata magnificens*), Kelp Gulls (*Larus dominicanus*), and Royal Terns (*Thalasseus maximus*) (Supplementary Table S1).

According to the IUCN (2025), all species are currently classified as Least Concern (LC), Brazilian national assessments, however, lists *S. magellanicus* as Near Threatened (NT) and *T. maximus* as Endangered (EN) in Brazil (ICMBio, 2018). Among the analyzed species, *S. leucogaster*, *L. dominicanus*, *F. magnificens*, and *T. maximus* are considered breeding residents in Brazil. Additionally, *T. maximus* is classified as a regular non-breeding visitor from both the South and the North, reflecting its migratory movements into Brazilian territory (Pacheco et al., 2021). *Spheniscus magellanicus*, in contrast, is a regular non-breeding visitor from southern regions, migrating annually from breeding colonies in Patagonia, Argentina, to the Brazilian coast during winter, sometimes reaching as far as the northeast (Stokes et al., 2014). All species included in this study occupy high trophic levels, relying primarily on fish as their main food resource, with some species showing preferences for food items such as cephalopods and crustaceans (Supplementary Table S1).

Sample preparation and laboratory analyses

Following dissections, muscle and liver tissues were frozen, freeze-dried for 48 h and homogenized. For metal and metalloid quantification, 100 mg aliquots were acid-digested with 1 mL of bi-distilled nitric acid (HNO₃, Hexis, 67% v/v) for 12 h, followed by heating at 100 °C for approximately 5 h. Digests were then brought to a final volume of 10 mL with ultrapure water. Elemental determinations were performed on a NexIon 300x ICP-MS (Perkin Elmer). Certified Reference Materials DORM-5 (fish muscle, NRC Canada) and NIST 2976 (mussel tissue, NIST, USA) were used for quality control. Their observed and certified values, along with recoveries, are provided in Supplementary Table S2. External calibration was conducted using a mixed standard solution (Merck IV, Sigma-Aldrich), and rhodium (Rh) served as the

internal standard. Instrumental Limits of Detection (LOD) and method Limits of Quantification (LOQ) were calculated following INMETRO (2020) guidelines (Supplementary Table S3).

Reduced glutathione (GSH) was quantified following Wilhelm-Filho et al. (2005). Briefly, samples were homogenized in sodium phosphate buffer (0.1 mol L⁻¹, pH 6.5) containing 0.25 mol L⁻¹ sucrose and 1 mmol L⁻¹ EDTA, then centrifuged at 11,000 × g for 30 min at 4 °C. The supernatants were mixed with phosphate buffer (0.1 mol L⁻¹, pH 8.0) containing 0.25 mmol L⁻¹ DTNB and incubated for 15 min. Absorbances were determined at 412 nm on an INNO microplate reader employing GSH as an external standard.

Metallothionein (MT) was extracted according to Erk et al. (2002), and quantified following Viarengo et al. (1997). Samples were homogenized in a Tris buffer (20 mmol L⁻¹, pH 8.6) containing 0.5 mmol L⁻¹ PMSF and 0.01% β-mercaptoethanol, then centrifuged at 20,000 × g for 60 min at 4 °C. The resulting supernatants were heated to 70 °C for 10 min and centrifuged again under the same conditions for 30 min. Final supernatants were reacted with a solution of 4 mmol L⁻¹ EDTA and 0.43 mmol L⁻¹ DTNB and incubated for 30 min. Absorbances were determined at 412 nm on an INNO microplate reader, with MT concentrations calculated using GSH as an external standard and applying a 1:20 MT:GSH molar stoichiometry (Linde and Garcia-Vazquez, 2006).

Statistical analyses

All statistical analyses were performed in the RStudio environment (version 4.5.1; R Core Team, 2025). Data normality was evaluated using the Shapiro–Wilk test. All variables violated normality assumptions, so non-parametric tests were applied. Spearman correlation coefficients were used to assess associations among variables, with correlation strengths interpreted according to Bryman and Cramer (2011). Interspecific differences in metal, metalloid, and biomarker concentrations were examined using the Kruskal–Wallis test followed by Dunn's *post hoc* test. Tissue-related differences (muscle vs. liver) were evaluated using the Mann–Whitney U test. Molar ratios concerning Se:Hg were calculated following Arisekar et al. (2024). All concentrations are reported in mg kg⁻¹ dry weight (d.w.).

Results

Metal and metalloid concentrations in seabirds

Metal and metalloid levels in the seabird muscle and liver samples are reported in Table 1. Percentages of samples below the LOQ are reported in Supplementary Table S4.

Concerning essential metals (Figure 2), *F. magnificens* presented the lowest Zn levels in liver and *S. leucogaster*, the highest in the same organ. *Sula leucogaster* also exhibited both the highest and lowest Zn and Cu concentrations in muscle. Copper and Zn in the five seabird species were not statistically different in both tissues. The highest Fe levels were found in *S. magellanicus* muscle and liver, and statistical differences were, with *S. magellanicus* differing from *F. magnificens*, *L. dominicanus*, and *S. leucogaster* in muscle, and from *L. dominicanus* and *S. leucogaster* in liver. Regarding different deposition between tissues, liver present higher depositions of these essential elements compared to muscle.

Concerning toxic elements (Figure 3), livers presented statistically higher Cd deposition compared to muscle, while no statistical differences between tissues were found for As and Pb. The highest Cd levels were found in both *S. magellanicus* liver and muscle, while *F. magnificens* exhibited the lowest levels in both tissues. Significantly higher Cd levels, in both tissues, were observed in *S. magellanicus* compared to *F. magnificens*, *L. dominicanus*, and *S. leucogaster*. In general, *S. magellanicus* and *S. leucogaster* presented high As levels in muscle and liver, respectively. No statistical differences concerning As in muscle were found between seabird species, while differences were noted for liver, with *S. leucogaster* differing from *F. magnificens* and *S. magellanicus*. The lowest Pb levels were found in both *S. leucogaster* muscle and liver, and the highest, in the liver of the same species. No interspecies statistical differences in Pb levels, however, were found.

Mercury is depicted separately, alongside Se in Figure 4. Regarding different deposition between tissues, liver present statistically higher values compared to muscle for both Hg and Se. The highest Hg concentrations were found in *S. magellanicus* muscle and *S. leucogaster* liver. The lowest Hg levels were observed in *S. leucogaster* muscle and in *F. magnificens* liver. No statistical differences were

found between species regarding Hg in liver, but muscle levels differed between *S. magellanicus* and *F. magnificens*, *L. dominicanus*, and *S. leucogaster*. For Se, the lowest concentrations were found in both tissues of *F. magnificens*, and the highest in *T. maximus* muscle and *S. leucogaster* liver. Moreover, *S. magellanicus* exhibited statistically significant higher concentrations of Se compared to the other species studied in both muscle and liver.

Regarding potentially toxic elements (Figure 5), statistically higher Ti levels were found in liver compared to muscle, with the highest and lowest concentrations found in *S. leucogaster* muscle and liver. Concentrations between species, however, were similar, with no statistical differences. Regarding Rb, no statistical differences between tissues were noted for this element. The highest Rb levels were found in *L. dominicanus* muscle and *S. leucogaster* muscle, and liver, while the lowest were observed in *F. magnificens* muscle and liver. Statistical differences for Rb were observed for muscle, with *S. magellanicus* differing from *F. magnificens* and *S. leucogaster*, and liver, with *F. magnificens* differing from *S. magellanicus* and *S. leucogaster*, as well as *S. magellanicus* differing from *S. leucogaster*.

Molar ratio between Se and Hg (Se:Hg)

Positive moderate correlations between Se and Hg were noted in both *S. magellanicus* muscle ($\rho = 0.463$) and liver ($\rho = 0.56$) and *S. leucogaster* muscle ($\rho = 0.589$) and liver ($\rho = 0.639$). Only four samples exhibited molar ratio Se:Hg < 1, one muscle sample (Se:Hg = 0.87), and three liver samples (Se:Hg = 0.76, 0.763, and 0.983), all from *S. leucogaster*.

Methalothionein and reduced glutathione determinations

The MT and GSH concentrations are presented in Supplementary Table S6. No statistical differences were found between species for either biomarker. However, concentration differences between tissues were observed, with higher concentrations observed in liver for both MT and GSH. Several moderate to very strong positive associations were observed between MT and GSH in both tissues of *S. magellanicus* and *S. leucogaster*, and in muscle of *F. magnificens* (Supplementary Table S7). Methalothionein was correlated with levels of Cd (*S. leucogaster* liver), Cu (*S.*

leucogaster muscle), Fe (*S. magellanicus* liver), Rb (*S. leucogaster* muscle), Se (*S. magellanicus* muscle), and Ti (*F. magnificens* and *S. leucogaster* muscle), while GSH was correlated with As (*S. leucogaster* muscle), Cd (*S. leucogaster* liver), and Ti (*S. leucogaster* muscle) (Supplementary Table S7).

Discussion

Essential metals

Concerning essential elements, liver concentrations were mostly higher than muscle levels, consistent with results commonly reported in the literature (Bahamonde et al., 2023). No differences between seabird species were noted for Cu and Zn, while Fe was found in higher levels in *S. magellanicus*. Concentration similarities of these elements between species are, however, expected, as they are essential to physiological process (Lima et al., 2023), likely suggesting typical levels for the analyzed species, although no baseline data are available on typical levels of these elements for the investigated seabird species.

For Zn, the used toxic thresholds for avian species is given as 2,100 mg kg⁻¹ d.w. (Varagiya et al., 2022). None of the seabirds investigated here presented medians or means (Table 1; Supplementary Table S5) above this value. This is likely due to Zn being an essential element and regulated by metabolic processes, and the fact that no serious sources of contamination of this element are noted along the coast of Rio de Janeiro (Lima et al., 2023). Furthermore, no correlations between Zn and biomarkers were found. The lack of correlations between GSH and Zn could be associated with low metal levels, not enough to induce GSH synthesis (Vizuite et al., 2023; Hernández-Moreno et al., 2021). Moreover, Zn was not correlated with MT, possibly due to the presence of this metal in diverse cellular compartments and not only associated with MT, acting in several metabolic process (Nam et al., 2005), or even as a consequence of high concentrations of other metals with a higher affinity for MT, such as Cd, Cu, and Hg, resulting in the release of Zn into the cellular environment (Jakimska et al., 2011).

Regarding Cu, used toxic thresholds vary from 9 to 28 mg kg⁻¹ d.w. in muscle and 367 mg kg⁻¹ d.w. in liver (Dolci et al., 2017), although this limit is not specific for seabirds. The muscle tissue analyzed herein presented medians and means (Table

1; Supplementary Table S5) above the lower limit ($> 9 \text{ mg kg}^{-1} \text{ d.w.}$), with 80% of muscle samples ranging from 9 to $28 \text{ mg kg}^{-1} \text{ d.w.}$ (two *T. maximus*, two *L. dominicanus*, three *F. magnificens*, 11 *S. leucogaster*, and 26 *S. magellanicus* samples). All liver showed medians and means (Table 1; Supplementary Table S5) below the toxic limit ($367 \text{ mg kg}^{-1} \text{ d.w.}$). This suggests that the observed levels in muscle tissues may be typical concentrations with no toxic effects. Copper is often reported as a major MT-inducer, and about 70 to 90% of this metal is observed bound to MT in liver of birds (*i.e.*, *Phalacrocorax carbo*, *Anas platyrhynchos*, *Anas poecilorhyncha*, *Anas acuta* and *Anas crecca*) (Nam et al., 2005). However, in the present study Cu and MT were correlated only in *S. leucogaster* muscle. The lack of correlations in liver combined with Cu values below toxic thresholds in this tissue can indicate values within physiological limits. On the other hand, all seabird species presented similar Cu values and above muscle thresholds but a Cu association with MT was observed only in *S. leucogaster*, suggesting that the toxic thresholds may be different between the five species and that, in *S. leucogaster*, the observed concentrations stimulate MT induction. Additionally, no correlation between Cu and GSH were found, probably due to low metal levels (Vizuite et al., 2023; Hernández-Moreno et al., 2021).

Specific thresholds for Fe in seabirds have not yet been established. Yet, the lack of correlations between GSH and Fe could be associated with levels not high enough to reflect on GSH synthesis and regeneration balance (Vizuite et al., 2023; Hernández-Moreno et al., 2021). The correlation between MT and Fe observed herein for *S. magellanicus* liver may be associated with the high levels of this metal detected in this species compared with the other seabirds, although no toxic threshold for Fe has been established to date. This suggests that MT is acting as a Fe regulator in the specie that present elevated concentrations of this metal.

Toxic metals

In the present study, As levels were similar between muscle and liver, this can suggest a possible remobilization to non-target tissues with lower metabolic rates, such as muscle. There is some uncertainty regarding As biomagnification, with reports of both negative (Linhares et al., 2024) and positive (Binkowski et al., 2024)

correlations between As levels and isotopic $\delta^{15}\text{N}$ values. Some authors suggest that this metalloid undergoes biodilution throughout food chains, although it is possible that As enrichment exhibits species-specific patterns (e.g., Sun et al., 2020), with accumulation more influenced by foraging area (i.e., background levels or contaminated areas) or type of prey (i.e., higher As levels in pelagic, benthic and demersal fish) than by trophic level (Correia et al., 2023; Lima et al., 2023; Anderson et al., 2010). In the seabirds analyzed herein, *S. magellanicus* and *S. leucogaster* showed the highest levels of As, likely due to habitat use of these species.

Some authors use As levels greater than 3 mg kg⁻¹ d.w. as associated with toxic effects in animals (Jerez et al., 2013). The medians of As for all investigated seabird species were below this value, while the mean concentration in *S. magellanicus* muscle (4.15 ± 6.68) was higher. Additionally, only three samples were above (one *S. leucogaster* liver, and two *S. magellanicus* muscle tissues). Moreover, many samples (66% in muscle, and 55% in liver; Supplementary Table S4) presented As concentrations below the LOQ (<0.018 mg kg⁻¹, d.w.). Furthermore, no correlations between As and MT was found, even though MT can act in the detoxification of As (Monteiro et al., 2019), the low concentrations of this metalloid may explain the lack of correlation with MT. Additionally, a positive correlation between As and GSH was found in muscle of *S. leucogaster*. Given that most As detoxification process involving GSH are expected to occur in liver, such as reduction of arsenate to arsenite, As methylation, and also in the formation of GSH-As complexes (Byeon et al., 2021), and that the correlation found between As and GSH was observed in muscle, is most likely that the levels of As are too low (below toxic thresholds and most below the LOQ) to trigger the use and reduction of GSH, as reported for other avian species (Sánchez-Virosta et al., 2020). This suggests that the analyzed organisms were exposed to background levels of this metalloid with no specific As contamination source. However, for a better understanding of this scenario, As speciation analyses should be conducted, due to the different toxicity degrees of organic and inorganic As (Byeon et al., 2021; Vizuite et al., 2018).

Cadmium levels were higher in liver than muscle of the seabirds analyzed here, as expected due to results commonly reported in the literature, usually associated to lower levels in non-target tissues, such as muscle (Lehel et al., 2023;

Scheuhammer, 1987). Some authors suggest that seabirds that inhabit pelagic/oceanic environments tend to exhibit higher levels of Cd than coastal, estuarine and terrestrial species (Lima et al., 2023), and some organisms typically exhibit high concentrations of this metal and are considered Cd sources, such as cephalopods (Linhares et al., 2024). In this sense, seabirds that consume high amounts of cephalopods have also been reported as routinely exhibiting high levels of this metal (Dolci et al., 2017; Anderson et al., 2010). In the present study, *S. magellanicus* exhibited significantly higher Cd levels, although high levels were also noted in *S. leucogaster*, probably due to the ingestion of high amounts of cephalopods by both (Mancini et al., 2023; Marques et al., 2018; Moura et al., 2018).

Several values have been used as toxic thresholds of Cd in the liver of avian species, such as $> 3 \text{ mg kg}^{-1} \text{ d.w.}$ (Scheuhammer, 1987), 40 to 60 $\text{mg kg}^{-1} \text{ d.w.}$ (Eisler, 1985), and even as high as 160 to 280 $\text{mg kg}^{-1} \text{ d.w.}$ (Kanstrup et al., 2019). The only species with liver median and means (Table 1; Supplementary Table S5) higher than one of these limits ($> 3 \text{ mg kg}^{-1} \text{ d.w.}$) was *S. magellanicus*. Furthermore, 45% of the analyzed liver samples were above this threshold (three *S. leucogaster*, and 13 *S. magellanicus* samples). Even though high levels of Cd were expected in *S. magellanicus* due to cephalopod consumption, this Cd concentrations are comparatively very high, which may result in potential toxicity. In the present study, positive correlations between GSH and Cd were observed in liver of *S. leucogaster*, the species that presented Cd concentrations above established toxic thresholds. This increased in GSH levels could also be associated with maintaining an equilibrium concerning synthesis and regeneration in its role as the first line of defense against oxidative stress (Srikanth et al., 2013). The association between MT and Cd is widely explored in the literature, and has been reported in avian species (Vanparys et al., 2008). Thus, the observed Cd-MT correlation in *S. leucogaster* liver was expected, due to MT being the main detoxification pathway of this metal (Vizuete et al., 2018; Nam et al., 2005; Scheuhammer, 1987). Some samples of *S. leucogaster* presented high Cd levels and the correlation with MT in liver indicates an adequate Cd detoxification process. In contrast, even though *S. magellanicus* presented medians and means higher than established thresholds and higher than in the other seabirds, no correlation was observed between Cd and the biomarkers, and

the lack of correlation especially with MT in this species, suggest a low capacity to detoxify these levels of Cd, which may lead to toxic effects.

Mercury was found in higher levels in hepatic tissue than in muscle, as expected due to Hg detoxification also occurs in liver (Bahamonde et al., 2023; Vizuite et al., 2018; Aazami, et al. 2011; Scheuhammer, 1987). This metal is well known to undergo biomagnification (Lemesle et al., 2024; Linhares et al., 2024; Sun et al., 2020; Bisi et al., 2012), directly affecting seabirds (Carravieri et al., 2014). Other factors, such as foraging area, have also been linked to Hg levels in seabirds, with higher concentrations found in pelagic/oceanic species (Lemesle et al., 2024; Lima et al., 2023). In addition, mesopelagic (Arisekar et al., 2024; Carravieri et al., 2014) and high trophic level (e.g., cephalopods; Becker et al., 2016; Carravieri et al., 2014) prey usually contain higher Hg levels, directly influencing Hg levels in seabirds. Herein, Hg was statistically higher in *S. magellanicus*, although *S. leucogaster* also presented high levels in some samples (including an outlier of 71.396 mg kg⁻¹ d.w.). Studies have reported high Hg levels in the prey items of these seabirds, such as cephalopods and anchovy (*Engraulis anchoita*) close to the study area (i.e., coastal area of the state of Rio de Janeiro) (Bisi et al., 2012), probably those dietary items are the cause of the high values found in *S. magellanicus* and *S. leucogaster*, who mainly feed on pelagic fish and cephalopods (Mancini et al.; 2023; Marques et al., 2018; Moura et al., 2018). The lower Hg contents of the other investigated seabirds (*F. magnificens*, *L. dominicanus*, and *T. maximus*) are probably due to their more frequent use of coastal environments and their generalist/opportunist feeding habits (Lemesle et al., 2024; Becker et al., 2016).

Mercury thresholds in muscle tissue are reported as 1.6 to 6.4 mg kg⁻¹ d.w. associated with moderate damage and reproductive effects (Ackerman et al., 2023; Zillioux et al., 1993), while levels > 17 mg kg⁻¹ d.w. have been linked to mortality (Zillioux et al., 1993) in some avian species. Herein, the medians and means (Table 1; Supplementary Table S5) of *T. maximus*, *S. leucogaster*, and *S. magellanicus*, and of 62% of the muscle samples (one *T. maximus*, two *F. magnificens*, six *S. leucogaster*, and 26 *S. magellanicus*) were above the lower limit (1.6 mg kg⁻¹ d.w.). Employed thresholds for avian species in liver are more numerous, such as > 4 mg kg⁻¹ d.w. (low/moderate damage and behavior effects; Ackerman et al., 2023; Zillioux

et al., 1993), $> 8 \text{ mg kg}^{-1} \text{ d.w.}$ (elevated damage and reproductive effects; Ackerman et al., 2023), and $> 20 \text{ mg kg}^{-1} \text{ d.w.}$ (severe damage and mortality; Ackerman et al., 2023; Zillioux et al., 1993). Among the analyzed seabirds, *S. leucogaster* and *S. magellanicus* exhibited medians and means (Table 1; Supplementary Table S5) above toxic limits (> 4 and $8 \text{ mg kg}^{-1} \text{ d.w.}$), with 52 % of liver samples exceeding some of the thresholds, *i.e.*, $4 \text{ mg kg}^{-1} \text{ d.w.}$ (six *S. magellanicus*, six *S. leucogaster*, and one *F. magnificens* samples), $> 8 \text{ mg kg}^{-1} \text{ d.w.}$ (three *S. leucogaster* samples), and $> 20 \text{ mg kg}^{-1} \text{ d.w.}$ (one *S. leucogaster* sample), probably due to dietary preferences, reflecting higher Hg concentrations in *S. leucogaster* and *S. magellanicus*.

Even though Hg levels were high, this metal was not correlated with GSH or MT. Nevertheless, Hg can be detoxified by other pathways, especially by formation of Se-compounds as Hg has a high affinity for Se-containing compounds, even higher than for molecules containing sulfhydryl groups, such as GSH and MT (Spiller, 2017; Scheuhammer, 1987). Selenium participates in the demethylation of MeHg, and formation of insoluble HgSe complexes (Spiller, 2017). In this sense, Se associations with Hg should be assessed to better understand Hg toxicity (Sobhakumari et al., 2018; Vizuite et al., 2018). In the present study, correlations were detected between these elements in the analyzed seabirds, strongly suggesting that Se is involved in Hg detoxification in the analyzed species. The use of Se and molecules containing this element (*e.g.*, certain amino acids, enzymes and proteins) by Hg detoxification mechanisms may lead to a deficiency or decreased availability of Se in exposed organisms (Spiller, 2017). Thus, the evaluation of Se:Hg molar ratios is paramount, with a protective effect occurring in cases of $\text{Se:Hg} > 1$ (Correia et al., 2023). The seabirds analyzed herein presented molar ratio $\text{Se:Hg} > 1$ on most samples, with the high Hg concentrations detected herein possibly representing this metal associated with Se in inert and insoluble complexes (Spiller, 2017). Four *S. leucogaster* samples, however, exhibited a molar ratio $\text{Se:Hg} < 1$, indicating potential toxicity, especially since the Hg levels in these samples were extremely high (*i.e.*, 5.22, 14.77, 14.71, and $71.40 \text{ mg kg}^{-1} \text{ d.w.}$) and above toxic thresholds, raising concerns regarding Hg seabird exposure and possible detrimental effects.

In the present study, Pb levels were similar between muscle and liver, however, this metal undergoes through other detoxification and accumulation mechanisms. Lead, in fact, has higher affinity for other tissues than liver and muscle in seabirds, such as bones and feathers, as this metal resembles calcium, and tends to accumulate in liver only when in higher concentrations (Bahamonde et al., 2023; Lehel et al., 2023; Sobhakumari et al., 2018; Scheuhammer, 1987). As the seabirds in this study all showed similar levels of this metal, exposure levels may be low in their habitats, although it is important to note that accumulation may take place in other, non-analyzed, tissues. Other studies have reported similar levels between other seabirds, as well as indeterminate patterns between species (Linhares et al., 2024; Lima et al., 2023). Furthermore, some authors suggest that Pb exhibits biodilution potential along the food chain (Binkowski et al., 2024; Linhares et al., 2024), and the similarity found here probably are due to the similar high trophic position between the investigated seabirds.

Some studies have used Pb toxic thresholds in liver for avian species, such as subclinical/sublethal effects ($> 6 \text{ mg kg}^{-1} \text{ d.w.}$; Sobhakumari et al., 2018), intoxication ($> 20 \text{ mg kg}^{-1} \text{ d.w.}$; Herring et al., 2017) and intoxication and severe effects ($> 60 \text{ mg kg}^{-1} \text{ d.w.}$; Herring et al., 2017). However, the medians and means of the seabirds analyzed herein were all much lower than the aforementioned limits. No correlations were found between Pb and the biomarkers, these low lead levels in the investigated species, with no samples above toxic limits, and no correlation with GSH, also suggesting levels not enough to cause increased antioxidant activity. Moreover, the absence of correlation between Pb and MT could be due to cellular distribution of this metal, in other cellular compartments rather than associated with MT, as reported in avian species by other authors (Vanparys et al., 2008), or due to its low concentrations observed in the analyzed seabirds.

Potentially toxic metals

In the present study Rb levels were similar between muscle and liver, and Ti showed higher levels in liver than muscle, however little is know about this metals distributions in tissues, due to scarcity of studies regarding these metals in seabirds (Bahamonde et al., 2023; Hauser-Davis et al., 2020). Some authors suggest

biomagnification of Rb (Padilha et al., 2021; Campbell et al., 2005), although no correlation with trophic level and foraging area has been reported in other studies (*i.e.*, Procelariiformes; Anderson et al., 2010). In the present study Rb was higher in *S. magellanicus*, a migratory species (Pacheco et al., 2021; Stokes et al., 2014), which could be associated with the use of different habitats and regions. Regarding Ti, studies conducted on the coast of Rio de Janeiro state have also reported high levels of Ti in several taxa, such as cetaceans (means higher than 14 mg kg⁻¹ d.w.; Monteiro et al., 2020) and cephalopods (means of approximately 8 mg.kg⁻¹ d.w.; Rodrigues et al., 2022). These data suggest that the coastal area of Rio de Janeiro poses a source of contamination of Ti (Monteiro et al., 2020), and that high trophic level organisms like cetaceans and seabirds may accumulate higher levels of this metal although scarce evidence in the literature about Ti biomagnification in general is available (Hauser-Davis et al., 2020; Asztemborska et al., 2018).

Specific thresholds of some elements, such as Rb and Ti, for seabirds have not yet been established. Herein, a positive association were observed between GSH and Ti. The increase (or at least reestablishment) of GSH levels after exposure to Ti (TiO₂-NP) has been reported in studies on freshwater fish (*i.e.*, *Prochilodus lineatus*; Carmo et al., 2018), mussels (*i.e.*, *Mytilus coruscus*; Huang et al., 2018), and zebrafish (*Danio rerio*; Ramsden et al., 2013), associated with an efficient antioxidant response and relevance on the role of GSH in this process. In this sense, even though no safe Ti concentration in seabirds has been established to date, a correlation between Ti and GSH was observed in *S. leucogaster* muscle, the species exhibiting high levels of this metal, suggesting control of the oxidant toxic effects of Ti. In the present study, positive correlations between MT and Rb in *S. leucogaster* muscle and Ti in both *S. leucogaster* and *F. magnificens* muscle were observed. In one assessment, Monteiro et al. (2020) reported associations between Ti and MT in cetaceans sampled from near the study area, suggesting that this protein may play a role in Ti detoxification. Since more than one seabird species exhibited this correlation (*S. leucogaster* and *F. magnificens*), the hypothesis of MT participation in the detoxification of Ti in seabirds seems valid, although further investigations are required. Regarding Rb, some authors indicate that this metal does not induce MT synthesis but can bind with pre-existing proteins in the cellular environment (Nam et

al., 2008; Nam et al., 2005). The positive correlation observed herein, however suggests that Rb may bind with MT as a detoxification mechanism, and the association of both only in *S. leucogaster* may be due to the presence of the extreme Rb values found in this species. This contrasts, however, with high Rb concentrations in *S. magellanicus* and a lack of correlation with MT, pointing to the need for further studies in this regard.

Biomarkers

Beyond the relevance of evaluating MT as a biomarker of metal exposure, given its well-documented capacity to bind and detoxify metals (Kehrig et al., 2016), some authors also propose that MT plays a role in antioxidant defense (Koivula and Eeva, 2010). In the present study, significant correlations between MT and GSH were observed in both liver and muscle tissues in multiple species (*i.e.*, *S. magellanicus*, *S. leucogaster*, and *F. magnificens*). These findings are consistent with previous observations in other organisms, such as cetaceans (*Pontoporia blainvillei* and *Sotalia guianensis*; Kehrig et al., 2016) and crabs (*Callinectes* sp.; Lavradas et al., 2014). The concordance between these biomarkers suggests that MT and GSH are similarly regulated and influenced by common environmental or physiological factors. Moreover, it reinforces the possibility that MT functions not only in metal detoxification but also as part of antioxidant defense mechanisms.

Interspecific MT induction variations may occur due to the different isoforms of this protein, depending on the species (Nam et al., 2008). However, in the present study no statistical differences were found between MT levels among species. Regarding tissue-specific differences in MT and GSH, higher concentrations of these biomarkers are generally expected in the liver due to its central role in metabolic activity and detoxification (Monteiro et al., 2019; Lavradas et al., 2014). Conversely, similar or high levels in muscle tissue may indicate substantial metal accumulation coupled with limited excretory capacity (Monteiro et al., 2020). In the present study, significant statistical differences were detected, with the liver exhibiting markedly higher concentrations of both biomarkers. These findings suggest that the seabirds analyzed herein are actively engaging hepatic metabolic processes aimed at toxicity,

attenuation and metal detoxification, while such processes appear less pronounced in muscle tissue.

Conclusion

Essential metal levels observed in the analyzed seabirds were consistent with expected baseline levels, as indicated by the similarity among species, values below established toxicity thresholds, and positive correlations with MT, suggesting effective metabolic regulation of these elements. Lead concentrations were low across all tissues and showed no correlation with either GSH or MT, indicating that the levels detected were insufficient to elicit an antioxidant or detoxification response. Although As levels were also low, the marked differences between species, particularly the high concentrations observed in *S. magellanicus* and *S. leucogaster*, highlight the need for further investigations, including detailed As speciation analyses.

Titanium exhibited significant correlations with both MT and GSH, suggesting active regulation by these biomarkers. These results highlight the importance of future studies evaluating the biomagnification potential of Ti and assessing whether coastal ecosystems of Rio de Janeiro may serve as a source of Ti exposure for seabird populations. High Rb concentrations were also detected in *S. magellanicus*, yet only *S. leucogaster* exhibited a correlation between Rb and MT, indicating that this migratory species may experience higher exposure accompanied by limited detoxification capacity.

Cadmium and Hg levels were higher in *S. magellanicus* and *S. leucogaster*. The absence of associations between Cd and detoxification biomarkers in *S. magellanicus* suggests reduced capacity for Cd detoxification, raising concerns about potential toxic effects and reinforcing the need for further research. Additionally, neither GSH nor MT seems to serve as the primary pathway for Hg detoxification, and Hg–Se interactions appear to dominate instead. While most individuals exhibited Se:Hg molar ratios over 1, some *S. leucogaster* specimens with the highest Hg burdens exhibited ratios below 1, suggesting increased vulnerability to Hg toxicity.

Overall, this study provides new insights into interspecific differences and antioxidant responses of seabirds exposed to metals, while also identifying critical knowledge gaps. Continued monitoring and comprehensive assessments of metal

and metalloid impacts on seabird health in the state of Rio de Janeiro are, thus, paramount to support effective conservation strategies.

Figures and Tables

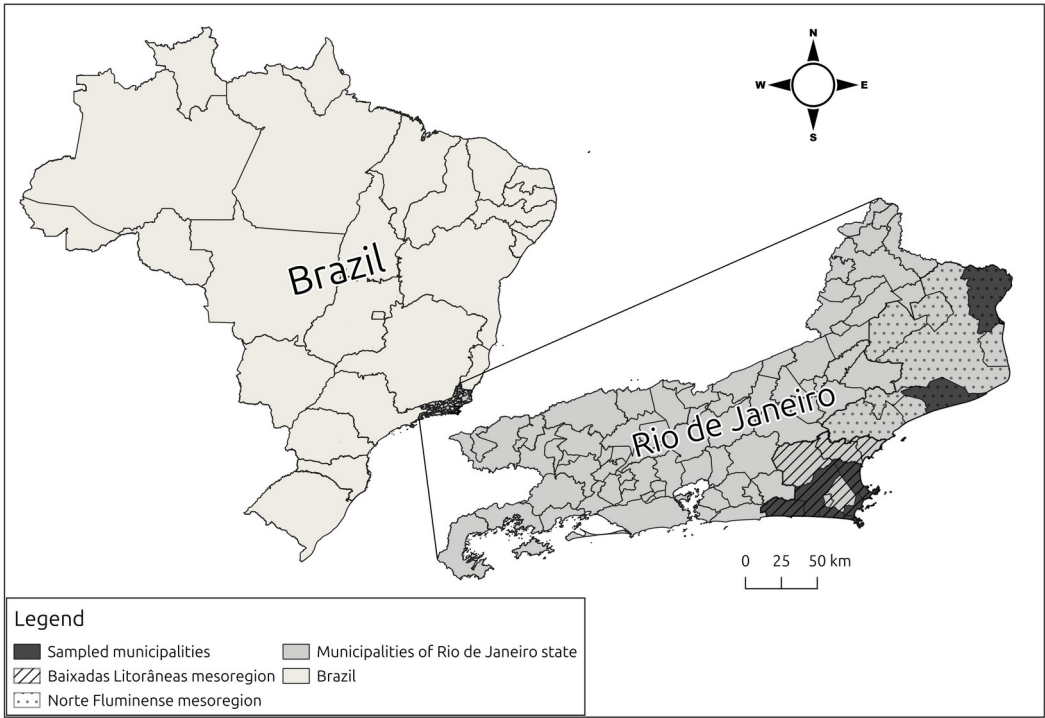


Figure 1. Seabird sampling locations in the state of Rio de Janeiro, Southeastern Brazil.

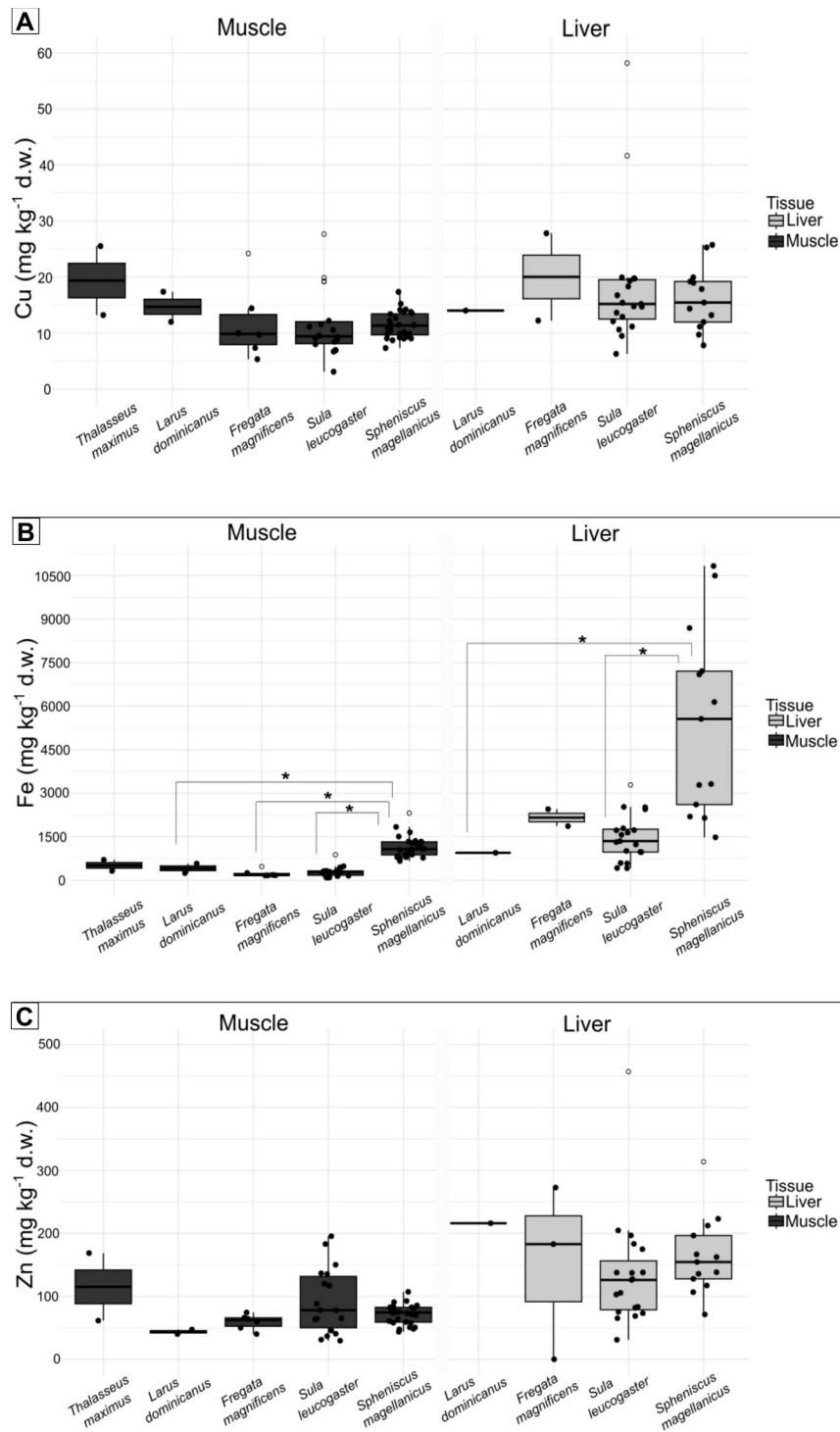


Figure 2. Essential metal concentrations (in mg kg⁻¹ d.w.) in the muscle and liver of the five seabird species analyzed herein sampled off the coast of Southeastern Brazil. (A) Cu, (B) Fe, (C) Zn. The central line in each boxplot is the median, the boxes indicate the interquartile range (IQR), upper and lower limits are indicated by

vertical lines, and outliers are noted as unfilled dots. Statistical differences ($p < 0.05$) between species are indicated by square brackets and asterisk (*).

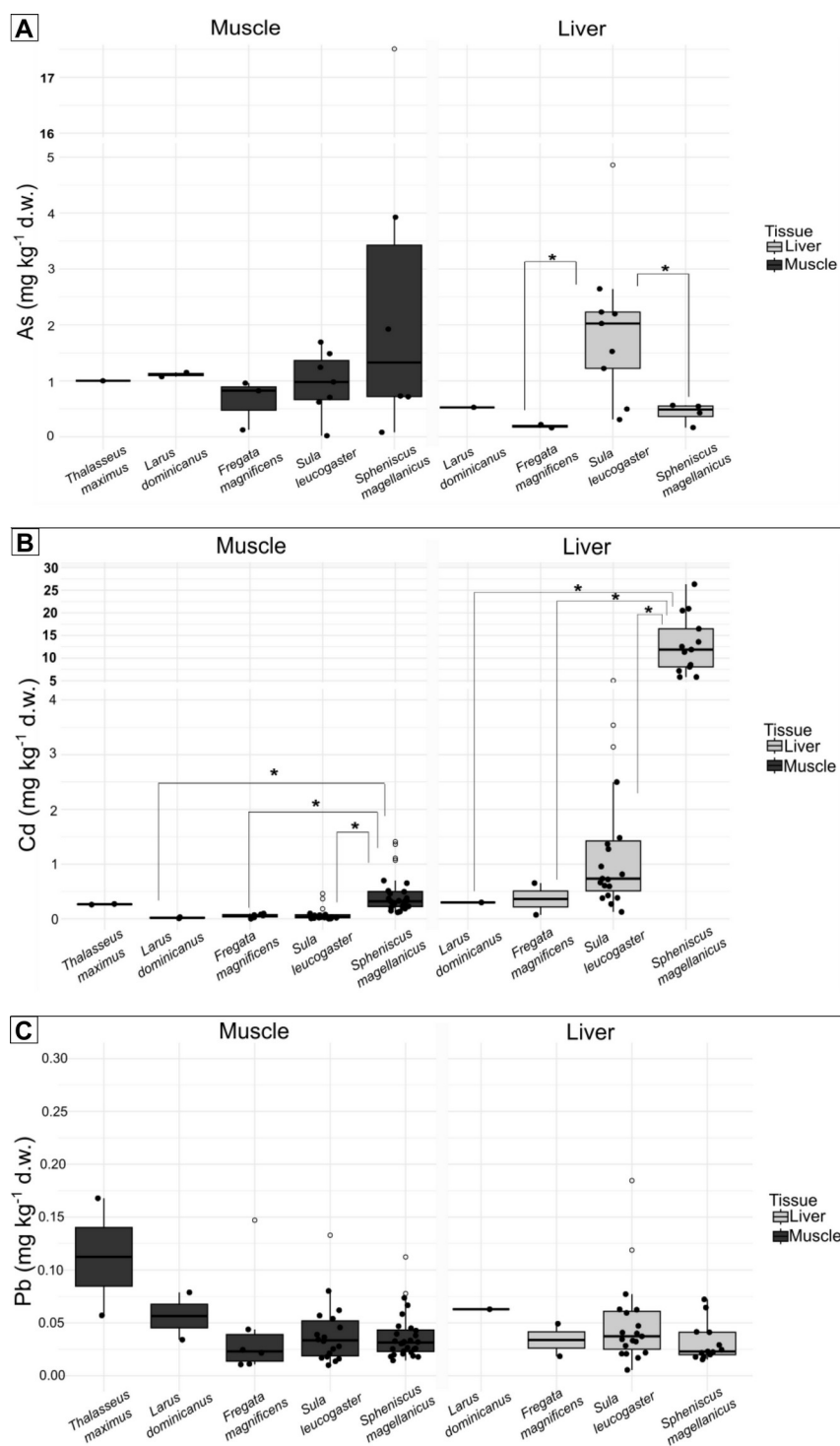


Figure 3. Toxic metal concentrations (in $\text{mg kg}^{-1} \text{ d.w.}$) in the muscle and liver of the five seabird species analyzed herein sampled off the coast of Southeastern Brazil.

(A) As, (B) Cd, (C) Pb. The central line in each boxplot is the median, the boxes indicate the interquartile range (IQR), upper and lower limits are indicated by vertical lines, and outliers are noted as unfilled dots. Statistical differences ($p < 0.05$) between species are indicated by square brackets and asterisk (*).

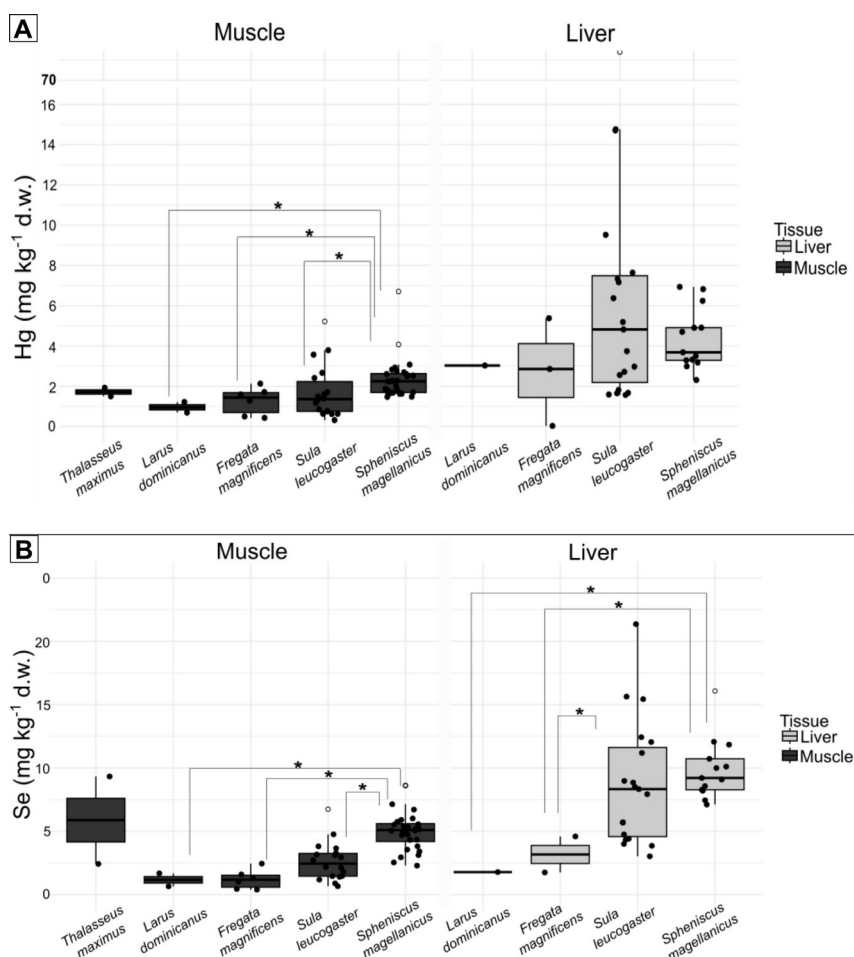


Figure 4. Mercury and selenium concentrations (in $\text{mg kg}^{-1} \text{ d.w.}$) in the muscle and liver of the five seabird species analyzed herein sampled off the coast of Southeastern Brazil. (A) Hg, (B) Se. The central line in each boxplot is the median, the boxes indicate the interquartile range (IQR), upper and lower limits are indicated by vertical lines, and outliers are noted as unfilled dots. Statistical differences ($p < 0.05$) between species are indicated by square brackets and asterisk (*).

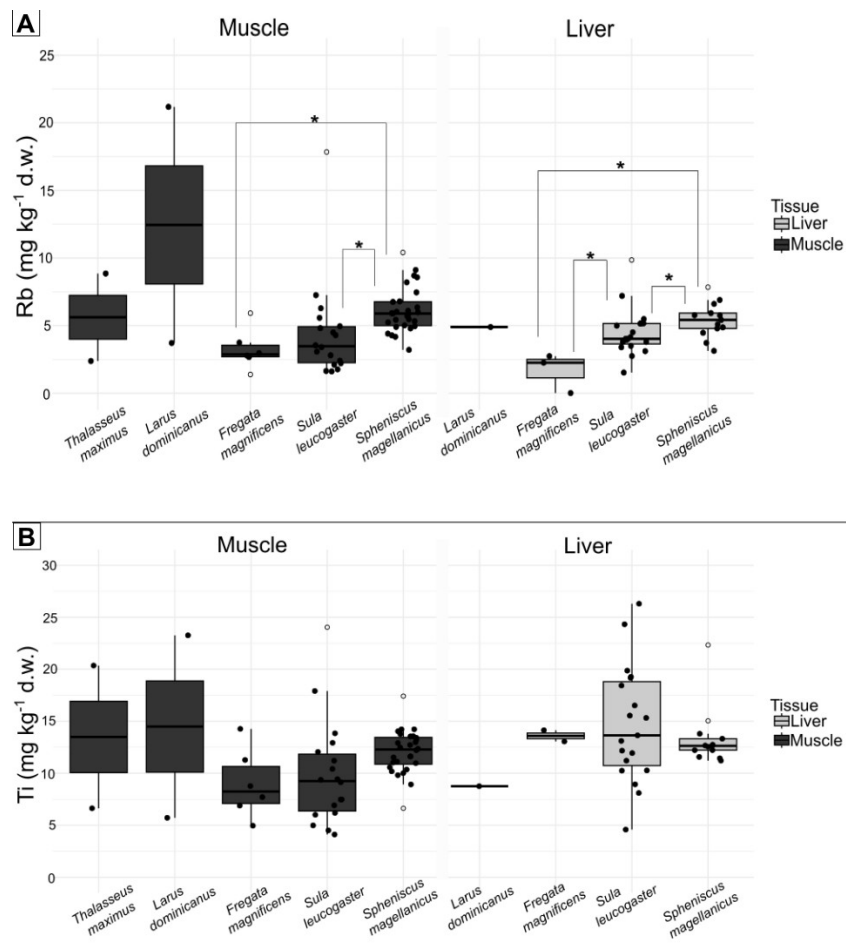


Figure 5. Potentially toxic metal concentrations (in mg kg⁻¹ d.w.) in the muscle and liver of the five seabird species analyzed herein sampled off the coast of Southeastern Brazil. (A) Rb, (B) Ti. The central line in each boxplot is the median, the boxes indicate the interquartile range (IQR), upper and lower limits are indicated by vertical lines, and outliers are noted as unfilled dots. Statistical differences ($p < 0.05$) between species are indicated by square brackets and asterisk (*).

Table 1. Metals and metalloid concentrations (mg kg⁻¹, d.w.) in seabird liver and muscle samples per species. Data are expressed as means $\pm$ standard deviations (minimal and maximal values).

<i>Fregata magnificens</i>		<i>Larus dominicanus</i>		<i>Spheniscus magellanicus</i>		<i>Sula leucogaster</i>		<i>Thalasseus maximus</i>
Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Muscle

As	0.188 ± 0.0422 (0.159 – 0.218)	0.637 ± 0.448 (0.126 – 0.961)	0.526 (0.526 – 0.526)	1.11 ± 0.0561 (1.07 – 1.15)	0.426 ± 0.184 (0.165 – 0.562)	4.15 ± 6.68 (0.0802 – 17.5)	1.95 ± 1.35 (0.311 – 4.86)	0.964 ± 0.571 (0.0199 – 1.69)	1.00 (1.00 – 1.00)
Cd	0.364 ± 0.410 (0.0737 – 0.654)	0.0570 ± 0.0350 (0.0042 – 0.0974)	0.300 (0.300 – 0.300)	0.021 ± 0.0153 (0.0099 – 0.0315)	13.0 ± 6.43 (5.81 – 26.3)	0.450 ± 0.361 (0.120 – 1.41)	1.32 ± 1.31 (0.128 – 5.01)	0.0877 ± 0.127 (0.0050 – 0.459)	0.267 ± 0.00863 (0.261 – 0.273)
Cu	20.0 ± 11.0 (12.2 – 27.8)	11.8 ± 6.78 (5.34 – 24.2)	14.0 (14.0 – 14.0)	14.7 ± 3.80 (12.0 – 17.4)	16.2 ± 5.60 (7.80 – 25.7)	11.4 ± 2.33 (7.32 – 17.3)	18.4 ± 12.0 (6.28 – 58.2)	11.6 ± 6.09 (3.10 – 27.7)	19.4 ± 8.70 (13.2 – 25.5)
Fe	2160 ± 414 (1870 – 2450)	236 ± 120 (165 – 472)	948 (948 – 948)	412 ± 231 (249 – 576)	5470 ± 3240 (1480 – 10800)	1150 ± 365 (665 – 2320)	1480 ± 792 (417– 3290)	300 ± 185 (92.0 – 880)	513 ± 273 (320 – 706)
Hg	2.76 ± 2.68 (0.0370 – 5.38)	1.28 ± 0.68 (0.436 – 2.13)	3.03 (3.03 – 3.03)	0.96 ± 0.37 (0.700 – 1.22)	4.37 ± 1.52 (2.32 – 6.94)	2.38 ± 1.04 (1.48 – 6.71)	8.91 ± 15.7 (1.57 – 71.4)	1.72 ± 1.33 (0.324 – 5.22)	1.72 ± 0.300 (1.51 – 1.93)
Pb	0.0338 ± 0.0218 (0.0184 – 0.0492)	0.0431 ± 0.0524 (0.0106 – 0.1471)	0.0629 (0.0629 – 0.0629)	0.0565 ± 0.0316 (0.0341 – 0.0788)	0.0316 ± 0.0183 (0.0153 – 0.0722)	0.0375 ± 0.0223 (0.0144 – 0.112)	0.0497 ± 0.0417 (0.0054 – 0.185)	0.0401 ± 0.0300 (0.0100 – 0.133)	0.112 ± 0.0784 (0.0570 – 0.168)
Rb	1.67 ± 1.46 (0.0076 – 2.74)	3.24 ± 1.52 (1.38 – 5.92)	4.89 (4.89 – 4.89)	12.4 ± 12.4 (3.71 – 21.2)	5.40 ± 1.28 (3.13 – 7.84)	6.10 ± 1.66 (3.20 – 10.4)	4.50 ± 1.77 (1.52 – 9.85)	4.44 ± 3.73 (1.61 – 17.8)	5.61 ± 4.58 (2.38 – 8.85)
Se	3.17 ± 2.01 (1.75 - 4.59)	1.21 ± 0.77 (0.391 - 2.45)	1.78 (1.78 - 1.78)	1.17 ± 0.73 (0.655 - 1.68)	9.90 ± 2.41 (7.11 - 16.1)	5.04 ± 1.55 (2.29 - 8.63)	8.76 ± 4.91 (3.03 - 21.4)	2.63 ± 1.52 (0.667 - 6.74)	5.88 ± 4.88 (2.43 - 9.33)
Ti	13.6 ± 0.764 (13.1 – 14.1)	8.98 ± 3.33 (4.95 – 14.3)	8.75 (8.75 – 8.75)	14.5 ± 12.4 (5.72 – 23.3)	13.4 ± 2.88 (11.2 – 22.3)	12.1 ± 2.07 (6.63 – 17.4)	14.7 ± 5.59 (4.58 – 26.3)	9.89 ± 5.05 (4.11 – 24.0)	13.5 ± 9.70 (6.63 – 20.4)
Zn	152 ± 139 (0.0990 – 273)	59.2 ± 12.3 (40.2 – 74.4)	216 (216 – 216)	43.7 ± 5.17 (40.1 – 47.4)	164 ± 62.1 (71.4 – 314)	71.8 ± 15.7 (43.7 – 107)	135 ± 91.6 (31.1 – 457)	92.2 ± 51.9 (29.7 – 196)	115 ± 75.9 (61.4 – 169)

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CRedit authorship contribution statement

LSP: Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. **TDSP:** Resources, Formal analysis, Writing – original draft, Writing – review & editing. **SS:** Supervision, Conceptualization, Resources, Data curation, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **RAHD:** Supervision, Conceptualization, Resources, Data curation, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing.

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Supplementary material

Table S1. Number of seabird individuals, number of muscle and liver samples, sampling areas, main food items and foraging habits per analyzed species. (n): number of samples or individuals; (NA): data not available.

Species	Number of tissue samples	Number of specimens per sampling area	Feeding characteristics		
			Main food	Foraging habits	Reference

items					
<i>Fregata magnificens</i>	Muscle (6) Liver (3)	Araruama (2); Arraial do Cabo (2); São Francisco de Itabapoana (1); NA (1)	Fish, cephalopods, crustaceans	Superficial diving, kleptoparasitism, fishing discards	Branco et al. (2007)
<i>Larus dominicanus</i>	Muscle (2) Liver (1)	Armação dos Búzios (1); Arraial do Cabo (1)	Fish, molluscs, crustaceans, insects	Generalist, opportunist, urban residues (garbage disposal), fishing discards	Marinao et al. (2018)
<i>Spheniscus magellanicus</i>	Muscle (28) Liver (13)	Araruama (27); NA (3)	Fish, cephalopods	-	Marques et al. (2018)
<i>Sula leucogaster</i>	Muscle (18) Liver (19)	Araruama (5); Armação dos Búzios (2); Arraial do Cabo (11); Cabo Frio (2); Quissamã (2); Saquarema (1); NA (2)	Fish	Opportunist, fishing discard	Mancini et al. (2023)
<i>Thalasseus maximus</i>	Muscle (2) Liver (0)	NA (2)	Fish, invertebrates	Fishing discards	Liechty et al. (2016)

Table S2: Certified and observed concentrations (mg kg⁻¹, dry weight – d.w.) in the DORM 5 (fish muscle) and NIST 2976 (mussel tissue) certified reference materials and their respective recovery rates.

Element	DORM 5		
	Certified value	Observed value	Recovery (%)
As	-	-	-
Cd	0.148	0.139 ± 0.022	93
Cu	3.300	2.881 ± 0.076	87
Fe	113	80.982 ± 3.758	71
Hg	0.316	0.372 ± 0.063	117
Pb	0.058	0.052 ± 0.005	88
Rb	2.760	2.27 ± 0.119	82

Se	2.400	1.712 ± 0.064	71
Ti	-	-	-
Zn	28.700	25.333 ± 0.424	88
NIST 2976			
Element	Certified value	Observed value	Recovery (%)
As	13.300	12.470 ± 0.351	93
Cd	-	-	-
Cu	4.020	3.875 ± 0.088	96
Fe	171.000	170.608 ± 17.519	99
Hg	0.061	0.073 ± 0.005	119
Pb	1.190	0.918 ± 0.038	77
Rb	-	-	-
Se	-	-	-
Ti	-	-	-
Zn	-	-	-

Table S3: Limits of Detection (LOD) and Limits of Quantification (LOQ) of the analyzed elements (mg kg⁻¹, d.w.).

Element	LOD	LOQ
As	0.0055	0.0180
Cd	0.0007	0.0020
Cu	0.0118	0.0400
Fe	0.1430	0.4800
Hg	0.0029	0.0100
Pb	0.0016	0.0050
Rb	0.0003	0.0010
Se	0.0891	0.3000
Ti	0.0252	0.0800
Zn	0.0209	0.0700

Table S4. Samples presenting metal and metalloid concentrations below the Limits of Quantification (mg kg⁻¹ d.w.). (n) number of individuals.

Element	LOQ	Tissue	Specie (n)	%
As	<0.018	Muscle	<i>T. maximus</i> (1); <i>F. magnificens</i> (3); <i>S. leucogaster</i> (11); <i>S. magellanicus</i> (22)	66
	<0.018	Liver	<i>F. magnificens</i> (1); <i>S. leucogaster</i> (10); <i>S. magellanicus</i> (9)	55
Cd	<0.002	Muscle	-	0
	<0.002	Liver	<i>F. magnificens</i> (1)	2
Cu	<0.04	Muscle	-	0
	<0.04	Liver	<i>F. magnificens</i> (1)	2
Fe	<0.48	Muscle	-	0
	<0.48	Liver	<i>F. magnificens</i> (1)	2
Hg	<0.01	Muscle	-	0
	<0.01	Liver	-	0
Pb	<0.005	Muscle	-	0
	<0.005	Liver	<i>F. magnificens</i> (1)	2
Rb	<0.001	Muscle	-	0
	<0.001	Liver	-	0
Ti	<0.08	Muscle	-	0
	<0.08	Liver	<i>F. magnificens</i> (1)	2
Zn	<0.07	Muscle	-	0
	<0.07	Liver	-	0

Table S5. Metal and metalloid concentrations (mg kg⁻¹, d.w.) in samples of liver and muscle of seabirds. (n) number of samples.

Arsenic

Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (2)	0.188	-
	Muscle (3)	0.825	-
<i>Larus dominicanus</i>	Liver (1)	0.526	-
	Muscle (2)	1.11	-
<i>Spheniscus magellanicus</i>	Liver (4)	0.488	-
	Muscle (6)	1.33	17.5
<i>Sula leucogaster</i>	Liver (9)	2.02	4.86
	Muscle (7)	0.980	-
<i>Thalasseus maximus</i>	Muscle (1)	1.00	-
Cadmium			
Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (2)	0.364	-
	Muscle (6)	0.0660	-
<i>Larus dominicanus</i>	Liver (1)	0.300	-
	Muscle (2)	0.021	-
<i>Spheniscus magellanicus</i>	Liver (13)	11.9	-
	Muscle (28)	0.322	1.07; 1.11; 1.36; 1.41
<i>Sula leucogaster</i>	Liver (19)	0.735	3.14; 3.54; 5.01
	Muscle (18)	0.0370	0.183; 0.367; 0.459
<i>Thalasseus maximus</i>	Muscle (2)	0.267	-
Cooper			
Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (2)	20.0	-
	Muscle (6)	9.85	24.2
<i>Larus dominicanus</i>	Liver (1)	14.0	-
	Muscle (2)	14.7	-

<i>Spheniscus magellanicus</i>	Liver (13)	15.4	
	Muscle (28)	11.3	-
<i>Sula leucogaster</i>	Liver (19)	15.2	41.7; 58.2
	Muscle (18)	9.41	19.2; 19.9; 27.7
<i>Thalasseus maximus</i>	Muscle (2)	19.4	-
Iron			
Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (2)	2160	-
	Muscle (6)	178	472
<i>Larus dominicanus</i>	Liver (1)	948	-
	Muscle (2)	412	-
<i>Spheniscus magellanicus</i>	Liver (13)	5560	-
	Muscle (28)	1070	2320
<i>Sula leucogaster</i>	Liver (19)	1350	3290
	Muscle (18)	284	880
<i>Thalasseus maximus</i>	Muscle (2)	513	-
Lead			
Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (2)	0.0338	-
	Muscle (6)	0.0230	0.147
<i>Larus dominicanus</i>	Liver (1)	0.0629	-
	Muscle (2)	0.0565	-
<i>Spheniscus magellanicus</i>	Liver (13)	0.0229	-
	Muscle (28)	0.0314	0.0777; 0.112
<i>Sula leucogaster</i>	Liver (19)	0.0373	0.119; 0.185
	Muscle (18)	0.0335	0.133
<i>Thalasseus maximus</i>	Muscle (2)	0.112	-
Mercury			

Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (3)	2.86	-
	Muscle (6)	1.44	-
<i>Larus dominicanus</i>	Liver (1)	3.03	-
	Muscle (2)	0.96	-
<i>Spheniscus magellanicus</i>	Liver (13)	3.69	-
	Muscle (28)	2.25	4.08; 6.71
<i>Sula leucogaster</i>	Liver (19)	4.83	71.4
	Muscle (18)	1.36	5.22
<i>Thalasseus maximus</i>	Muscle (2)	1.72	-
Rubidium			
Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (3)	2.25	-
	Muscle (6)	2.87	1.38; 5.92
<i>Larus dominicanus</i>	Liver (1)	4.89	-
	Muscle (2)	12.4	-
<i>Spheniscus magellanicus</i>	Liver (13)	5.42	7.84
	Muscle (28)	5.89	10.4
<i>Sula leucogaster</i>	Liver (19)	4.02	9.85
	Muscle (18)	3.47	17.8
<i>Thalasseus maximus</i>	Muscle (2)	5.61	-
Selenium			
Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (2)	3.17	-
	Muscle (6)	1.18	-
<i>Larus dominicanus</i>	Liver (1)	1.78	-
	Muscle (2)	1.17	-

<i>Spheniscus magellanicus</i>	Liver (13)	9.21	16.1
	Muscle (28)	5.10	8.59; 8.63
<i>Sula leucogaster</i>	Liver (19)	8.33	-
	Muscle (18)	2.45	6.74
<i>Thalasseus maximus</i>	Muscle (2)	5.88	-
Titanium			
Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (2)	13.6	-
	Muscle (6)	8.24	-
<i>Larus dominicanus</i>	Liver (1)	8.75	-
	Muscle (2)	14.5	-
<i>Spheniscus magellanicus</i>	Liver (13)	12.6	15.0; 22.3
	Muscle (28)	12.3	6.63; 17.4
<i>Sula leucogaster</i>	Liver (19)	13.6	-
	Muscle (18)	9.25	24.0
<i>Thalasseus maximus</i>	Muscle (2)	13.5	-
Zinc			
Species	Tissue (n)	Median	Outliers
<i>Fregata magnificens</i>	Liver (3)	183	-
	Muscle (6)	62.3	-
<i>Larus dominicanus</i>	Liver (1)	216	-
	Muscle (2)	43.8	-
<i>Spheniscus magellanicus</i>	Liver (13)	155	314
	Muscle (28)	74.5	-
<i>Sula leucogaster</i>	Liver (19)	126	457
	Muscle (18)	78.1	-
<i>Thalasseus maximus</i>	Muscle (2)	115	-

Table S6. Concentrations methalothionein and reduced glutathione levels in samples of liver and muscle of seabirds (in $\mu\text{mol g}^{-1}$ d.w.). (n) number of samples; (SD) standard deviation; (Range) minimum and maximum values.

Methalothionein			
Tissue (n)	Median	Mean $\pm$ SD	Range
Liver (35)	140.36	289.90 $\pm$ 309.48	51.33 – 1379.44
Muscle (57)	117.01	201.95 $\pm$ 220.96	49.48 – 956.67
Reduced glutathione			
Tissue (n)	Median	Mean $\pm$ SD	Range
Liver (35)	3.12	5.73 $\pm$ 6.45	1.41 – 30.95
Muscle (57)	2.52	3.73 $\pm$ 3.83	0.90 – 20.44

Table S7. Statistically significant correlations between metallothionein (MT), reduced glutathione (GSH), and the metals and metalloids analyzed herein in the muscle and liver of seabirds sampled off the coast of southeastern Brazil.

Association	Species	Tissue	ρ	Strength
MT x GSH	<i>Fregata magnificens</i>	Muscle	0.886	Strong
	<i>Spheniscus magellanicus</i>	Liver	0.616	Moderate
		Muscle	0.427	Moderate
	<i>Sula leucogaster</i>	Liver	0.950	Very strong
		Muscle	0.598	Moderate
MT x Cd	<i>Sula leucogaster</i>	Liver	0.584	Moderate
MT x Cu	<i>Sula leucogaster</i>	Muscle	0.511	Moderate
MT x Fe	<i>Spheniscus magellanicus</i>	Liver	0.610	Moderate
MT x Rb	<i>Sula leucogaster</i>	Muscle	0.410	Moderate
MT x Se	<i>Spheniscus magellanicus</i>	Muscle	0.362	Weak
MT x Ti	<i>Fregata magnificens</i>	Muscle	0.829	Strong

	<i>Sula leucogaster</i>	Muscle	0.496	Moderate
GSH x As	<i>Sula leucogaster</i>	Muscle	0.750	Strong
GSH x Cd	<i>Sula leucogaster</i>	Liver	0.534	Moderate
GSH x Ti	<i>Sula leucogaster</i>	Muscle	0.414	Moderate

Supplementary References

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DISCUSSÃO GERAL

Após levantamento bibliográfico através de revisão sistemática e quantitativa (de Paula et al., 2025), apresentada no Anexo I, foi possível observar que fígado e penas são as matrizes mais utilizadas em estudos de monitoramento de metais em aves no Brasil. Além disso, dentre as 37 espécies analisadas no Brasil, aves costeiras e marinhas representam o maior foco das pesquisas. Dentre os elementos mais reportados em trabalhos científicos com aves no Brasil estão Cd, Cr, Cu, Hg, Pb e Zn. Tendo em vista que entre os metais analisados em músculo e fígado de aves marinhas no presente estudo encontram-se Cd, Cu, Hg, Pb e Zn, certas comparações podem ser feitas a respeito de concentrações destes elementos em fígado em diferentes espécies, locais e estudos.

No presente estudo, as cinco espécies de aves marinhas analisadas apresentaram medianas similares de Cu (medianas entre 10 e 20 mg kg⁻¹ peso seco – p.s.) e Zn (medianas variando de 100 a 200 mg kg⁻¹ p.s.), esses valores foram próximos dos reportados nos estudos conduzidos no Brasil, com estimativas de concentrações médias (*i.e.*, *pooled estimate*) de 16,87 mg kg⁻¹ p.s. de Cu, e de 91,07 mg kg⁻¹ p.s. de Zn (Anexo I). Além disso, outros autores reportaram valores similares de Cu para algumas das espécies analisadas neste estudo, como *S. leucogaster*, *L. dominicanus* e *F. magnificens* (*e.g.*, Moura et al., 2018). É de suma importância que níveis seguros destes elementos para tecidos de aves sejam estabelecidos, a fim de facilitar o monitoramento de possíveis contaminações por Cu e Zn. Devido ao fato de nenhuma das espécies analisadas no presente estudo apresentar concentrações que ultrapassem os limites tóxicos para Cu e Zn em fígado, além da similaridade destes metais entre espécies e estudos, possivelmente esses valores representem concentrações típicas para esses elementos em fígado de aves.

Chumbo foi um metal encontrado em baixas concentrações, tanto na revisão de trabalhos científicos (estimativas de concentrações médias – *pooled estimate* de 0,37 mg kg⁻¹ p.s.) (Anexo I), quanto nas cinco espécies de aves marinhas analisadas (mínimo de 0,005 e máximo de 0,185 mg kg⁻¹ p.s.). Assim como outros estudos conduzidos com *S. magellanicus* no estado do Rio de Janeiro também relataram baixas concentrações de Pb (Kehrig et al., 2015; Vega et al., 2010). Com relação ao Hg, as estimativas de concentrações médias (*i.e.*, *pooled estimate*) dos trabalhos

conduzidos no Brasil foi de 0,64 mg kg⁻¹ p.s. (Anexo I), no entanto valores elevados deste metal foram reportados em alguns estudos (e.g., > 10 mg kg⁻¹ p.s., Costa et al., 2023; Oliveira et al., 2023). Nas aves marinhas analisadas no presente estudo, as concentrações de Hg em fígado apresentaram grande variação intraespecífica, no entanto, a menor e maior mediana de concentração de Hg foram encontradas em *F. magnificens* (2,861 mg kg⁻¹ p.s.) e *S. leucogaster* (4,826 mg kg⁻¹ p.s.) respectivamente. É possível observar certa proximidade nas concentrações de Hg encontradas no presente estudo e no restante do Brasil quando se tratando de aves marinhas, como no caso de medianas de Hg de 3,69 mg kg⁻¹ p.s. em *S. magellanicus* neste estudo, e demais estudos com valores de média entre 3,7 e 5,70 mg kg⁻¹ p.s. (Kehrig et al., 2015; Vega et al., 2010). Estudos conduzidos utilizando penas como matriz na investigação de Hg em *S. leucogaster* (Bighetti et al., 2022; Nunes et al., 2022; Bighetti et al., 2021) reportaram concentrações mais elevadas próximo da costa de Macaé, RJ (i.e., Arquipélago de Santana) quando comparado a locais mais afastados (i.e., Arquipélago de Abrolhos e Trindade). Além disso, no presente estudo conduzido na costa do estado do Rio de Janeiro, fígado de indivíduos de *S. leucogaster* apresentaram elevadas concentrações de Hg (compatíveis com possíveis efeitos tóxicos e baixa capacidade de destoxificação), esse cenário reforça a susceptibilidade desta espécie à contaminação por Hg e possivelmente elevas concentrações ambientais deste metal no estado do Rio de Janeiro.

A tendência de concentrações de Cd em aves no Brasil também foi similar ao observado no presente estudo. As estimativas de concentrações médias (i.e., *pooled estimate*) de Cd em fígado de aves foi de 0,26 mg kg⁻¹ p.s. (Anexo I), no entanto, valores mais elevados foram reportados em alguns estudos realizados com *S. magellanicus* onde a média de concentrações de Cd chegou a 24,40 mg kg⁻¹ p.s. (Kehrig et al., 2015; Vega et al., 2010). No presente estudo, os níveis de Cd foram geralmente baixos, com exceção de *S. magellanicus* que apresentou concentrações mais elevadas quando comparada às demais espécies, com mediana de 11,868 mg kg⁻¹ p.s. e variação de valores entre 5,805 e 26,327 mg kg⁻¹ p.s.. Esses dados reforçam que aves marinhas que consomem grande quantidade de cefalópodes, como *S. magellanicus*, estão expostas a elevadas concentrações de Cd (Dolci et al.,

2017; Anderson et al., 2010), sendo necessário maior monitoramento desses animais.

CONCLUSÃO GERAL

A análise de concentrações de metais e metaloides em tecidos de aves marinhas, combinada ao levantamento bibliográfico sistemático e quantitativo do uso de aves como biomonitores destes contaminantes no Brasil, sugere certas tendências no comportamento destes elementos em aves. Isto reforça algumas considerações e hipóteses sobre ambos o status de contaminação e os possíveis riscos para algumas espécies. De maneira geral, os metais essenciais apresentaram concentrações consistentes entre espécies e estudos, sugerindo que esses valores possam ser utilizados como “concentrações típicas” em futuros estudos de monitoramento, tendo em vista que valores seguros ou tóxicos para aves marinhas são escassos. Além disso, as aves marinhas analisadas apresentaram comumente baixas concentrações tanto de As, quanto de Pb, assim como no restante de estudos conduzidos no Brasil.

Em linhas gerais, as concentrações de Cd e Hg encontradas neste estudo seguiram a tendência reportada no restante da produção científica no Brasil, onde *S. magellanicus* e *S. leucogaster* são as espécies mais vulneráveis e susceptíveis à contaminação por estes metais. Mesmo que os níveis de Cd e Hg tenham sido similares à outros trabalhos, as associações com os biomarcadores MT e GSH, além da razão molar Se:Hg investigadas aqui, indicam que indivíduos destas espécies possam apresentar risco de desenvolverem efeitos deletérios devido a exposição à esses metais. Neste cenário, destacam-se os casos onde *S. magellanicus* apresentou as maiores concentrações de Cd e falta de correlação com biomarcadores, e *S. leucogaster* com amostras apresentando concentrações extremamente elevadas de Hg juntamente com razão molar Se:Hg < 1.

Elevadas concentrações de contaminantes emergentes (Rb e Ti) foram encontradas nas aves marinhas analisadas, apontando o litoral do estado do Rio de Janeiro como uma região potencial de contaminação por Rb e Ti. Embora ainda não existam limites tóxicos definidos para Rb e Ti, suas correlações com biomarcadores de estresse oxidativo e destoxificação (GSH e MT) indicam que esses metais podem desencadear respostas fisiológicas relacionadas ao controle do efeitos deletérios causados por estes metais em aves marinhas. Desta forma os achados deste estudo

reforçam a necessidade de futuras investigações a respeito de efeitos tóxicos e o comportamento de Rb e Ti nos compartimentos ambientais.

Este estudo aponta possíveis valores típicos de metais essenciais (Cu, Fe e Zn), além de espécies mais susceptíveis, e com possível risco toxicológico, à contaminação por Cd e Hg. Ademais, sugere que o litoral do estado do Rio de Janeiro apresenta elevadas concentrações de contaminantes emergentes (Rb e Ti). Esses resultados são de suma importância para guiar futuros estudos de monitoramento sobre contaminação de metais e metaloides e aves marinhas no estado do Rio de Janeiro, além de apresentar subsídio com dados científicos que demonstram a necessidade de que estratégias de mitigação sejam desenvolvidas.

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ANEXO I

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A SYSTEMATIC REVIEW AND QUANTITATIVE SYNTHESIS OF METAL AND METALLOID CONTAMINATION IN AVIAN SPECIES IN BRAZIL

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Abstract

Avian species are well-suited as sentinel species concerning metal and metalloid contamination, although knowledge on this subject in Brazil is still scarce and scattered in the literature. In this sense, this review, conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) methodology by means of keyword searches across the Web of Science, Scopus, and PubMed databases, aimed to compile information on this subject in Brazil. In focusing on Brazil, we address the country's unique biodiversity, diverse ecosystems, and specific anthropogenic pressures, while consolidating scattered and regionally published data to support targeted biomonitoring and conservation efforts. Concentrations of key metals (Cd, Cr, Cu, Hg, Pb, Zn) in different bird tissues in Brazil were estimated. Feathers and liver are the most frequently investigated tissues, with the former exhibiting high potential in biomonitoring assessments as a non-invasive sampling method. The most common analytical methods were Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Cold Vapour Atomic Absorption Spectroscopy (CVAAS), and Atomic Absorption Spectroscopy. *Ardea alba*, *Sula leucogaster*, *Larus dominicanus*, and *Spheniscus magellanicus* are the most studied species. Metal concentrations were varied throughout different studies, indicating the need for standardized protocols and long-term monitoring to track contamination trends and assess mitigation efforts.

Keywords: Birds, sentinel species, Brazil; metal and metalloid contamination.

Highlights

- Birds are ideal sentinel species for monitoring metal contamination.
- In Brazil, feathers and liver are the most studied tissues
- Common metals analyzed in birds include Cd, Cr, Cu, Hg, Pb, and Zn.
- ICP-MS, CVAAS, and AAS are the most used methods for metal analysis in birds.
- Standardized protocols and long-term monitoring are needed to track contamination.

1. Introduction

Metal and metalloid mobilization occurs naturally through geochemical processes, although anthropogenic activities such as industrial activities, agricultural practices, mining, and expansion of urbanization have increased their environmental deposition [1-4]. Diverse environmental matrices are now contaminated by metals and metalloids, such as soil [1, 3, 5, 6], water [7, 8], sediment [2, 7, 9, 10], air [11], and biota [7, 12, 13].

Long-term exposure of living organisms to metals and metalloids can result in a range of direct and indirect adverse effects. Indirect effects include alterations of dietary resource in contaminated areas, which may ultimately result in reduced breeding success in some animals, such as birds [14], while direct effects comprise various deleterious physiological [15], behavioral [16], and reproductive [17] effects. Thus, metal and metalloid assessments are paramount in biodiversity conservation efforts, preferably comprising long-term analyses [18, 19]. This is an important strategy for monitoring the presence and potential effects of these contaminants in the environment and in animal populations. Monitoring efforts also contribute to health risk assessments [3, 20-22], and the prediction of future damage [23], enabling mitigation strategies [3, 22].

Sentinel and bioindicator species are routinely employed to detect environmental contaminants, and may provide insights on pollutant bioavailability, uptake, and effects [23]. Various organisms have been noted as potential bioindicators [23], such as algae [24], plants [8, 13], invertebrates [25, 26], fish [12], reptiles [27], birds [28], and mammals [29]. Nevertheless, differential exposure levels and routes concerning contaminants among species are noted, due to environmental factors such as soil or water bioavailability [6, 23, 30], and species-specific characteristics like behavior, trophic level, foraging habitats, geographical distribution, migration patterns, and longevity [18, 23, 31-34].

Animals near or at the top of their respective food chains, particularly in aquatic environments, are more susceptible to bioaccumulation and biomagnification processes concerning different metals and metalloids, as the case of mercury, zinc, and lead [33, 36-38]. Birds occupy different trophic levels and are exposed to differential contaminant levels [31, 32, 34], with those at the top of the food chain

comprising suitable bioindicators in environmental contamination assessments [32, 37, 39].

The most studied metals and metalloids in avian species are As, Cd, Hg, Pb and Se, with Hg being the most frequently assessed [34]. Arsenic and selenium are commonly classified as metalloids due to their intermediate physical and chemical properties between metals and non-metals [40]. The USA is the most prolific country regarding scientific publications on the subject, at 24.72% of the global percentage [39]. However, when focusing on seabirds, countries in the Arctic region have published more extensively, followed by some European countries, while South American countries such as Brazil, even though comprising an avian biodiversity hotspot, still represents a small percentage of the global scientific production in this regard [34]. Thus, we chose to focus this review exclusively on Brazil, due to multifold reasons. Brazil is home to one of the richest avifaunas in the world, including numerous endemic and threatened species, making it highly relevant to evaluate contaminant exposure within this unique ecological context. The country encompasses diverse biomes—such as the Amazon, Pantanal, Cerrado, and Atlantic Forest—each subjected to distinct anthropogenic pressures, including mining, agriculture, urbanization, and artisanal gold extraction, all of which are potential sources of metal and metalloid pollution. Despite this, available data on avian contamination in Brazil remain scarce, fragmented, and often limited to regional publications, many in Portuguese, which restricts their accessibility in global assessments. A focused national review allows for the consolidation of this dispersed literature, identification of critical knowledge gaps, and promotion of standardized biomonitoring protocols tailored to the Brazilian context. Moreover, localized assessments are essential for informing national environmental policy, conservation planning, and long-term risk evaluation efforts.

To fill this knowledge gap, a systematic review and quantitative synthesis was carried out aiming at obtaining a comprehensive perspective on studies employing avian species as sentinels and bioindicators for metal and metalloid contamination in Brazil. The most studied species and preferred sampled matrices are also discussed. The findings may be used to direct future monitoring projects in Brazil, extensible to other countries worldwide.

2. Material and methods

2.1. Data collection and selection

This study was conducted in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) methodology [41]. Data were retrieved in the first semester of 2024, through keyword searches across three major academic databases, namely Web of Science, Scopus, and PubMed. Only scientific papers were selected, while other productions such as theses, dissertations, and abstracts were not included. To broadly cover the literature, the language and publication date of the papers were not elimination factors. The aim was to identify publications regarding the use of avian species as sentinels and bioindicators concerning metal and metalloid assessments in Brazil.

The keyword search was formulated to include a range of elements pertinent to the subject matter, including the geographical focus on Brazil, the use of avian species, and the investigation of metals and metalloids. The following array of keywords was used: (bird OR seabird OR waterbird OR avian OR "marine bird" OR "coastal bird") AND (contamination OR pollution OR bioaccumulation OR biomagnification OR "environmental contamination" OR "environmental pollution" OR "contaminant levels" OR "metal concentrations" OR "heavy metal exposure" OR "metalloid exposure" OR "toxic metal levels" OR "toxic metalloid levels" OR "environmental monitoring" OR biomonitoring OR "trace element accumulation" OR "trace metal accumulation" OR "anthropogenic pollution" OR "human-induced contamination" OR "heavy metal contamination" OR "metalloid contamination") AND (metal OR metalloid OR "trace element" OR mercury OR Hg OR cadmium OR Cd OR lead OR Pb OR arsenic OR As OR chromium OR Cr OR nickel OR Ni OR copper OR Cu OR zinc OR Zn) AND (Brazil OR Brazilian).

The number of documents retrieved from each database was recorded to establish the extent and distribution of relevant literature across different platforms. The resulting publications were retrieved and compiled for further analysis. The raw data was analyzed in the R environment for duplicate elimination. Eligible documents were selected for the systematic review and quantitative synthesis through a screening process conducted in accordance with established criteria. Inclusion criteria comprised studies investigating metals and metalloids, conducted in Brazil

and assessing avian species, while exclusion criteria encompassed studies that investigated other contaminants, studies not conducted in Brazil, that did not employ avian species, and studies with insufficient data on the subject.

2.2. Data analysis

The retrieved data were analyzed using the R version 4.4.1 [42]. Data frequency was considered to create visual representations and construct tables for a scientometric analysis, including the most studied elements, the main studied bird species, analytical techniques, as well as the most studied Brazilian regions, using the *ggplot2* (v3.5.1) [43], *sf* (v1.0.16) [44, 45], *dplyr* (v1.1.4) [46], *readr* (v2.1.5) [47], and *tidyr* (v1.3.1) [48] packages.

Forest plots were constructed using the *dplyr* (v1.1.4) [46], and *ggplot2* (v3.5.1) [43] packages, based on a weighted mean (e.g. pooled estimate). The Confidence Interval (CI) of 95% was calculated with a critical value (α) of 0.05. The differences between the means of each study and the pooled means were then calculated. When the SD was not provided in the original studies, the R software was employed to estimate this error. Additionally, the R software was used to estimate a single mean and SD for studies that presented these data in a fragmented manner (see *Supplementary Material*).

Most papers presented the values of element concentrations in dry weight, although some presented data in wet weight [49-52]. Thus, to compare studies, a conversion was applied by assuming 70% of water content in the liver, as water content levels in bird livers typically range between 70% and 75% [52-55]. All values of concentrations of metals and metalloids are presented in dry weight (d.w.).

3. Results and discussion

3.1. Study selection

Following duplicate elimination, 213 records were identified within the three searched databases, reduced to 40 after the screening process. The studies were then selected for two purposes, a systematic review and a quantitative synthesis (Figure 1).

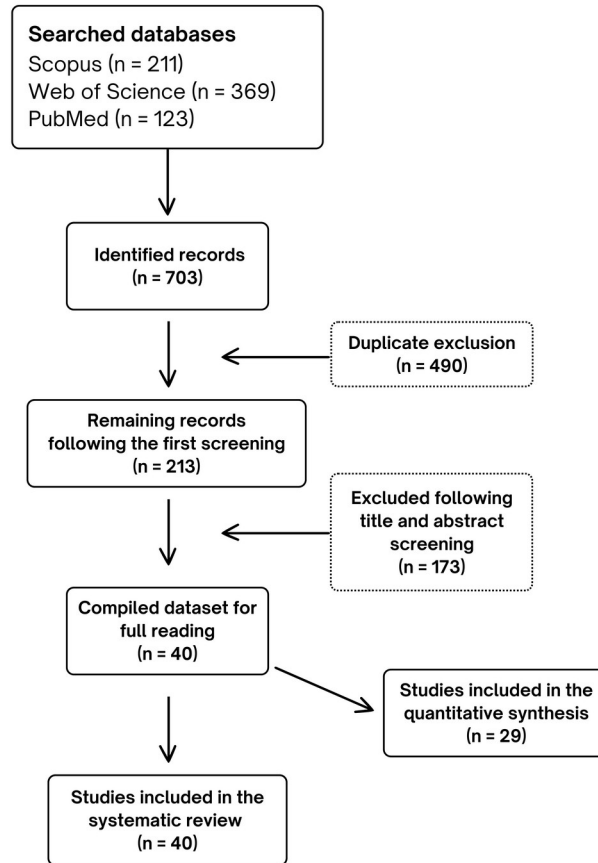


Figure 1. Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow diagram applied to this review. Adapted from Page et al. [41].

Seven of the retrieved studies were reviews and were used only in the systematic review [56-62]. Study selection included in the quantitative synthesis include only data regarding concentrations in tissues from studies that report the most frequently analyzed elements (*e.g.*, large number of studies) in the most commonly used matrices. This criterion allowed for comparisons concerning only elements in tissues cited in a considerable number of publications. Furthermore, only concentration data for adult individuals were compared, given that bird age can significantly influence metal and metalloid levels [63-67]. In addition, differences between male and female birds were not considered, as most studies do not report these variables. Finally, we considered differences in sample preparation protocols specifically in cases where variations could significantly influence the interpretation of results, due to the potential for external contamination.

Four articles investigating metals and metalloids in birds in Brazil were included exclusively in the systematic review. These studies either examined different matrices (blood) [68], focused on juvenile individuals [69, 70], or lacked sufficient data [71].

3.2. Metals and metalloids in avian species in Brazil

In addition to the review studies, a total of 33 studies were identified as part of the scientific production in Brazil regarding the investigation of metals and metalloids in birds. The most frequently analyzed elements include Hg (22 studies), Cd (20 studies), Pb and Zn (17 studies), Cu (16 studies), and chromium (Cr) (15 studies) (Figure 2).

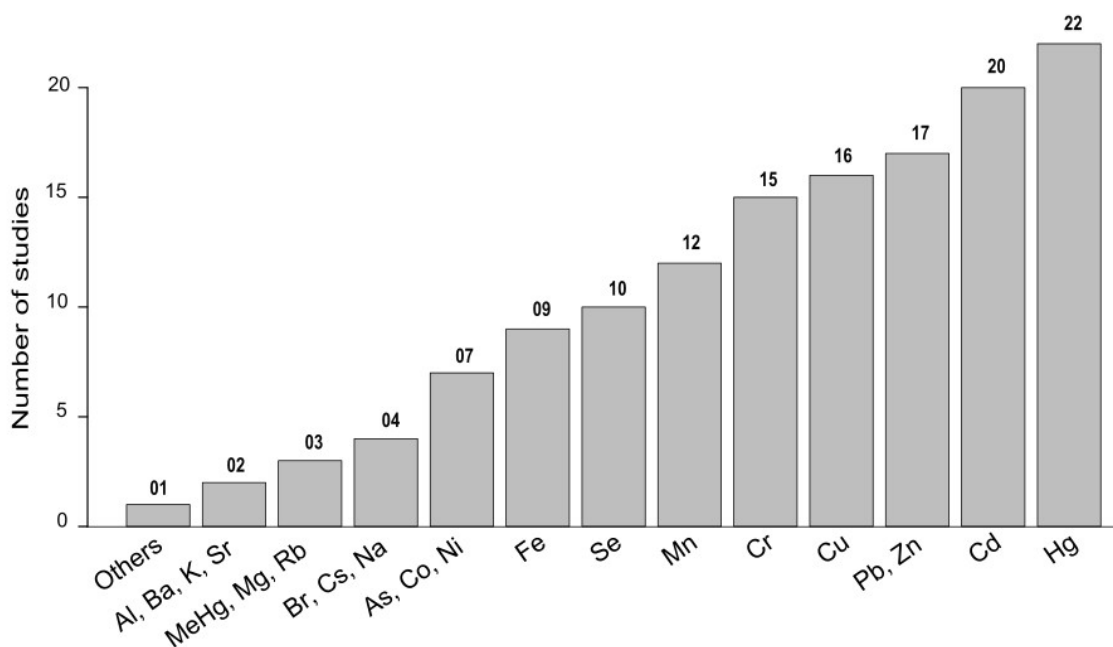


Figure 2. Number of studies investigating metals, and metalloids in avian species published in Brazil. The columns with grouped elements represent the same number of studies for each element. The column “Others” represent elements of a single publication each, namely Ca, Ce, Cl, Hf, La, Mo, Sc, Sb, Sm, Sn, Tb, Th, Yb, and V.

Regarding the temporal distribution of Brazilian publications (see *Supplementary Material*), the first retrieved paper was published in 1997 [62], and the most recent in 2024 [72]. After 2009, at least one study was published per year. In general, the number of publications increased over time, beginning between 2010-

2011 (three studies each year), with peaks in 2018 (five publications), and in 2022, comprising the highest number of published studies (seven).

The complete map displaying the total number of publications per state in Brazil is presented in *Supplementary Material*. The regions with the highest amounts of publications on metals and metalloids in avian species are states located in the Brazilian southeast (Espírito Santo (ES), Rio de Janeiro (RJ), and São Paulo (SP)), and south (Paraná (PR), Santa Catarina (SC), and Rio Grande do Sul (RS)) Brazil. Fifteen studies were published in the southeast, while the south accounted for ten publications. The major research focus in these regions was on seabirds (*Spheniscus magellanicus*, and *Sula leucogaster*), and *Ardea alba*. Additionally, the corresponding biomes are the Atlantic Rainforest and Pampa (only in RS).

A considerable literature on metals and metalloids is available in northern Brazil, specifically in the Amazon biome. In this context, the main focus has been on Hg use in artisanal and small-scale gold mining (ASGM) [73-76], especially investigating soil [77, 78], water [79, 80], and fish [81, 82] as the main matrices, due to their perceived direct relevance to human health and local economies, such as traditional communities [83-85]. Our results, thus, highlight that avian species are not routinely employed as environmental contamination sentinels in this biome. All Amazon states, including Mato Grosso (MT), which is part of the Brazilian Legal Amazon but also includes the Cerrado and Pantanal biomes, accounted only for five studies [49-51, 86, 87].

Another understudied biome on this topic is the Cerrado, with two studies [49, 88]. This is consistent with the major research focus in this biome being associated with plant biodiversity [89]. However, recent studies have demonstrated that climate change and changes in land use are likely to impact avian Cerrado biodiversity in the near future [90, 91]. One of the main concerns is agriculture expansion within this biome [92, 93], which is known for increasing metal and metalloid levels in the environment by the use of chemicals (fertilizers and pesticides) [94-97]. Therefore, it is crucial that future research in Cerrado focuses on investigating metal contamination in avian species to provide a broader view of the ecological challenges faced within this biome.

Twenty distinct analytical techniques were employed in the retrieved studies (Table 1). The most frequently applied were Atomic Absorption Spectroscopy (AAS), Cold Vapour Atomic Absorption Spectroscopy (CVAAS), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), totaling six studies each. These highly sensitive and accurate techniques enable multi-element analyses, with low limits of detection requiring relatively low sample volumes ([98-100], both significant advantages for metal and metalloid research.

Table 1. Number and percentage of studies employing distinct analytical techniques for the investigation of metals and metalloids in avian species in Brazil.

Analytical Technique	Number of studies	%
Atomic Absorption Spectroscopy (AAS)	6	18
Cold Vapour Atomic Absorption Spectroscopy (CV-AAS)	6	18
Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	6	18
Flame Atomic Absorption Spectrometry (FAAS)	4	12
Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS)	4	12
CV-AAS coupled with Flow Injection Analysis System/Mercury System (CV-AAS-FIAS)	3	09
Instrumental Neutron Activation Analysis (INAA)	3	09
Atomic Fluorescence Spectroscopy (AFS)	2	06
Cold Vapour Atomic Fluorescence Spectroscopy (CV-AFS)	2	06
GFAAS with High-Resolution Continuum Source (GF-AAS-CS)	2	06
Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)	2	06
AAS with Gold Amalgamation (AAS-GA)	1	03
Electrothermal Atomic Absorption Spectrometry (ET-AAS)	1	03
Flameless GF-AAS	1	03
Gas Chromatograph coupled to an Atomic Fluorescence Spectrometer (GC-AFS)	1	03

Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES)	1	03
k0-INAA	1	03
Vapour Generation Atomic Absorption Spectrometry (VG-AAS)	1	03
Total	33	100

Certain variations were observed with regard to the different analytical techniques employed for the most extensively analyzed elements (Hg, Cd, Cr, Cu, Pb, and Zn) (Table 2).

Table 2. Number of studies that utilized different analytical techniques for the investigation of the most analyzed elements (Cd, Cr, Cu, Hg, Pb, and Zn) in birds in Brazil.

Analytical Technique	Hg	Cd	Pb	Zn	Cu	Cr
AAS	2	4	4	3	3	2
CV-AAS	6	-	-	-	-	-
ICP-MS	3	5	3	4	3	3
FAAS	1	3	3	3	3	3
GF-AAS	1	1	2	2	1	2
CV-AAS-FIAS	3	-	-	-	-	-
INAA	1	1	-	3	2	-
AFS	2	-	-	-	-	-
CV-AFS	2	-	-	-	-	-
GF-AAS-CS	1	2	2	2	2	2
ICP-OES	-	2	1	-	1	1
AAS-GA	1	-	-	-	-	-
ET-AAS	-	1	-	-	1	-
Flameless GF-AAS	-	1	1	-	-	1
GC-AFS	1	-	-	-	-	-
ICP-AES	-	1	1	1	1	1
k0-INAA	1	-	-	1	-	1

VG-AAS	1	-	-	-	-	-
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A total of 37 bird species were investigated (Table 3). Most are classified as “Least Concern - LC” by the IUCN [101], although some are categorized as “Near Threatened - NT” (e.g. *Calidris pusilla* and *Calidris subruficollis*), “Vulnerable - VU” (e.g. *Procellaria aequinoctialis*, *Procellaria conspicillata*, and *Pterodroma arminjoniana*), and “Endangered - EN” (e.g. *Thalassarche chlororhynchos*) [101]. One study evaluated metals and metalloids levels in *Columba livia* [88], an exotic bird that has been introduced in Brazil [102]; this species has a large distribution and abundance in urbanized areas with proximity to humans [103], which could be useful for the assessment of contamination in these areas.

A variety of feeding habits and trophic niches of the investigated species are noted, including carnivores/generalists (e.g., *Tyto furcata* [104]), scavengers (e.g., *Coragyps atratus* [105]), insectivores (e.g., *Basileuterus flaveolus* [106]), and granivores (e.g., *Arremon flavirostris* [106]). Nevertheless, the most frequent were piscivorous, generalists, and opportunistic species (e.g., *Ardea alba*, *Nannopterum brasilianum*, *Sula leucogaster*, and *Thalasseus acuflavidus* [107-109], consistent with the fact that most of the utilized species in the retrieved studies are aquatic, coastal, or seabirds.

Table 3. Number of studies for each investigated avian species.

Order	Species	H g	C d	Pb	Zn	C u	Cr	Othe r	References
Apodiformes	<i>Amazilia lactea</i>	1	0	0	1	0	1	1	[71]
Cathartiformes	<i>Coragyps atratus</i>	2	0	0	1	0	1	1	[51, 71]
Charadriiformes	<i>Calidris pusilla</i>	1	1	1	0	0	1	1	[68]
	<i>Calidris subruficollis</i>	0	1	1	1	1	1	1	[110]
	<i>Larus dominicanus</i>	2	2	2	3	2	3	2	[69, 111, 112]

	<i>Thalasseus acuflavidus</i>	1	1	0	1	1	1	1	[111]
Ciconiiformes	<i>Mycteria americana</i>	1	0	0	0	0	0	0	[86]
Columbiformes	<i>Columba livia</i>	0	1	1	1	1	1	1	[88]
	<i>Leptotila verreauxi</i>	1	0	0	1	0	1	1	[71]
Coraciiformes	<i>Chloroceryle amazona</i>	1	0	0	0	0	0	0	[49]
	<i>Chloroceryle americana</i>	1	0	0	0	0	0	1	[50]
	<i>Megaceryle torquata</i>	1	0	0	0	0	0		[49]
Falconiformes	<i>Caracara plancus</i>	1	1	1	0	1	1	0	[113]
Passeriformes	<i>Arremon flavirostris</i>	1	0	0	1	0	1	1	[71]
	<i>Basileuterus flaveolus</i>	1	0	0	1	0	1	1	[71]
	<i>Synallaxis spixi</i>	1	0	0	1	0	1	1	[71]
Pelecaniformes	<i>Ardea alba</i>	5	2	0	4	2	1	4	[51, 71, 87, 114-116]
	<i>Ardea cocoi</i>	1	0	0	0	0	0	0	[51]
	<i>Cochlearius cochlearius</i>	1	0	0	0	0	0	0	[51]
	<i>Egretta thula</i>	1	1	1	1	1	1	1	[51, 117]
Phaethontiformes	<i>Phaethon aethereus</i>	2	2	2	2	2	2	2	[72, 118]
Procellariiformes	<i>Procellaria aequinoctialis</i>	1	1	1	1	1	0	0	[119]
	<i>Procellaria</i>	1	1	1	1	1	0	0	[119]

	<i>conspicillata</i>								
	<i>Pterodroma arminjoniana</i>	1	1	1	1	1	1	1	[118]
	<i>Puffinus gravis</i>	0	1	1	0	1	0	1	[120]
	<i>Puffinus puffinus</i>	1	1	1	0	0	0	1	[121]
	<i>Thalassarche chlororhynchos</i>	1	1	1	1	1	1	1	[122]
	<i>Thalassarche melanophris</i>	1	1	1	1	1	1	1	[122]
Sphenisciformes	<i>Spheniscus magellanicus</i>	3	2	2	0	0	0	1	[52, 70, 123]
Strigiformes	<i>Asio stygius</i>	0	1	1	0	0	1	1	[124]
	<i>Megascops spp.</i>	0	1	1	0	0	1	1	[124]
	<i>Pulsatrix koeniswaldiana</i>	0	1	1	0	0	1	1	[124]
	<i>Tyto furcata</i>	0	1	1	0	0	1	1	[124]
Suliformes	<i>Anhinga anhinga</i>	1	0	0	0	0	0		[51]
	<i>Fregata magnificens</i>	1	2	0	1	2	1	2	[111, 125]
	<i>Nannopterum brasilianus</i>	2	1	1	1	1	1	1	[51, 122]
	<i>Sula leucogaster</i>	5	5	2	4	5	3	6	[72, 111, 118, 125-128]

Upon examination of the most extensively analyzed elements, seven species were employed in multiple studies for at least one element (Table 3). As indicated by Celik et al. [39], the most extensively researched avian taxa worldwide encompass Ardeidae, Anatidae, Laridae, and Accipitridae. This aligns with the trends observed in Brazilian studies, where *A. alba* is representative of the Ardeidae taxon and *Larus*

dominicanus is representative of the Laridae taxon. Additionally, it is important to note that the Suliformes order is a prominent subject in Brazilian studies, with species such as *Fregata magnificens*, *Nannopterum brasilianum*, and *Sula leucogaster* being frequently utilized.

Five of the most extensively analyzed species are seabirds (*F. magnificens*, *L. dominicanus*, *Phaethon aethereus*, *Spheniscus magellanicus*, and *S. leucogaster*), and the other two are continental, but also inhabit coastal environments (*A. alba*, and *N. brasilianum*). These species occupy relatively high trophic levels, and are usually piscivorous, but also exhibit generalist and opportunistic feeding habits [107, 108, 129-132]. *Ardea alba* and *N. brasilianum* exhibit a wide distribution across Brazil, and the remaining seabirds are widely distributed along the Brazilian coast (except for *P. aethereus*). These features make these species an adequate choice as bioindicators. The studies investigating the most commonly researched metals analyzed eleven different tissues (Table 4). Among these, muscle, blood, and kidney were subjected to frequent analysis, although liver and feathers were the two most utilized tissues.

Table 4. Number of studies employing different tissues as biological matrices for investigation of the most analyzed elements in avian species Brazil.

Tissue	Cd	Cr	Cu	Hg	Pb	Zn
Liver	11	5	7	12	8	6
Feather	11	9	11	14	9	9
Kidneys	4	2	1	2	4	1
Blood	4	4	4	7	5	4
Muscle	3	1	1	6	2	2
Heart	1	1	-	1	1	-
Eggs	1	-	1	1	-	1
Brain	-	-	-	1	-	-
Lung	-	-	-	1	-	-
Claws	-	-	-	1	-	-
Rhamphotheca (beak epidermis)	-	-	-	1	-	-

Vizuete et al. [34] reported that the levels of certain elements (Hg, Cd, and Pb) generally follow a similar order of magnitude, with higher levels observed in the liver compared to kidneys and feathers. In general, metals and metalloids accumulate predominantly in tissues involved in detoxification processes, such as liver and kidneys, and in feathers, which are an important excretion route for some metals and metalloids [39]. The use of feathers as a biomonitoring tissue has, in fact, become increasingly prevalent due to its non-invasive nature [39, 56, 57, 133, 134]. Other non-invasive matrices, like eggshells, have also been widely used in this sense worldwide, due to the maternal transfer of these elements during egg formation [19, 127, 135].

3.3. Quantitative synthesis concerning the most analyzed elements in avian species in Brazil

To reduce potential bias, selected papers used in the quantitative synthesis were restricted to those investigating the six most analyzed elements (Hg, Cd, Pb, Zn, Cu, and Cr) in the two most utilized matrices (liver and feathers) in adult bird individuals.

The comparison of metal and metalloid levels in the tissues of different avian species is a challenging undertaking, largely due to the influence of interspecific factors on the contaminant accumulation [31, 32, 34]. However, due to the limited number of studies examining specific elements in the same species within Brazil, the mean differences in the levels of the most analyzed elements were initially analyzed collectively across all species and regions by the construction of forest plots. After this initial assessment, studies were then compared by area (Brazilian biomes, states, or regions) and species (same species, genera, or trophic niche).

3.3.1. Mercury (Hg)

Pooled estimates for Hg concentrations in the livers and feathers of Brazilian birds were quite similar ($0.64 \mu\text{g g}^{-1}$ and $1.12 \mu\text{g g}^{-1}$, respectively) (Figure 3). Only one study concerning liver concentrations [114] and two regarding feather concentrations [72, 87] exhibited a CI crossing the pooled estimate line, suggesting that Hg concentrations do not differ from the global average of the studies included; this

could be due to their weights on the pooled estimate or similarity between reported concentrations.

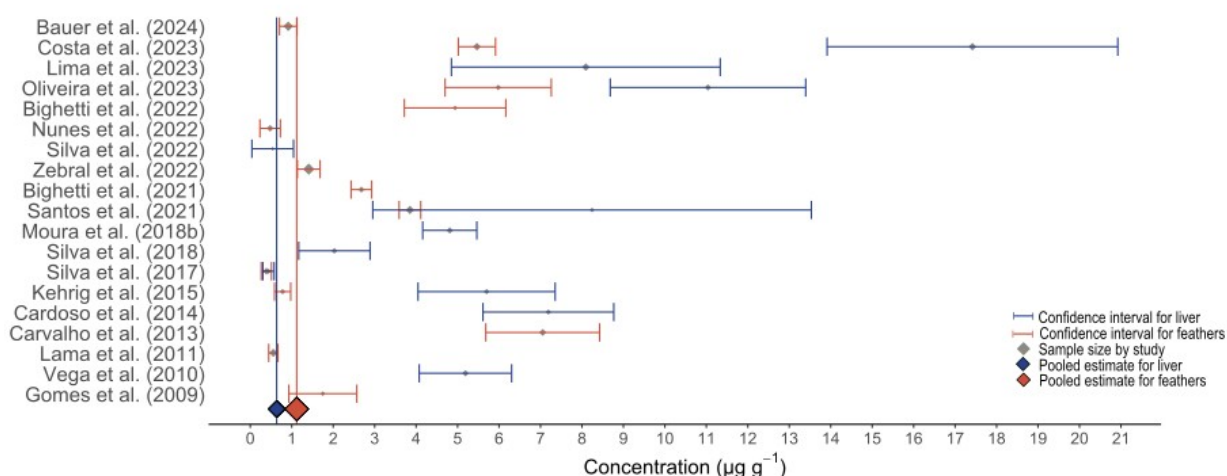


Figure 3. Forest plot for mercury, with pooled estimates for both liver ($0.64 \mu\text{g g}^{-1}$) and feathers ($1.12 \mu\text{g g}^{-1}$) in avian species analyzed in Brazil. The error bars correspond to a Confidence Interval (CI) of 95%.

Some reports present levels below the pooled estimate in feathers (e.g. [86, 113, 118, 123]), and one study in liver [113]. The remaining studies revealed higher means, both in liver [49-52, 111, 115, 121-123], and in feathers [49-51, 119, 127, 136]. Some of these levels are close to or even higher than $5 \mu\text{g g}^{-1}$, the concentration in feathers known to be associated with sublethal and reproductive damage in birds [137, 138]. Some of those high concentrations were reported by Oliveira et al. [50], with found Hg levels in liver ($11.04 \pm 7.85 \mu\text{g g}^{-1}$) and in feathers ($5.98 \pm 3.17 \mu\text{g g}^{-1}$) in *Chloroceryle americana*. Similarly, Costa et al. [49] reported liver and feather values of $10.63 \pm 5.97 \mu\text{g g}^{-1}$ and $6.96 \pm 2.11 \mu\text{g g}^{-1}$ respectively in *Megasceryle torquata*.

In some cases, only slight variations in Hg concentrations are observed between studies using the same species and matrix. For example, Hg concentrations in liver of *S. magellanicus* were $5.70 \pm 3.73 \mu\text{g g}^{-1}$ in the state of Rio Grande do Sul [123], while Vega et al. [52] reported $5.7 \pm 4.1 \mu\text{g g}^{-1}$ in the state of Rio de Janeiro, and $3.7 \pm 2.1 \mu\text{g g}^{-1}$ in Rio Grande do Sul. Within this context, the closeness of means found between studies can be due to both the analyzed individuals of the

state of Rio Grande do Sul (southern Brazil) and especially due to the migration patterns of this species.

Another example of variability between studies using the same species is verified for *A. alba* liver samples from the Madeira River in the state of Amazonas, in the city of São Paulo and in the metropolitan area and cities in the state of São Paulo (Figure 4). Even though these are all distant regions presenting different potential contamination sources, levels of mercury ($2.92 \mu\text{g g}^{-1}$) in the Madeira River [51] were similar to those observed in state of São Paulo ($2.20 \pm 2.37 \mu\text{g g}^{-1}$) and in the city of São Sebastião ($2.20 \pm 0.21 \mu\text{g g}^{-1}$) [115]. Lower values, however, have been reported for some metropolitan regions of the same state ($0.54 \pm 0.40 \mu\text{g g}^{-1}$) [114] and in the city of Sorocaba ($0.55 \pm 0.39 \mu\text{g g}^{-1}$) [115]. It is important to highlight that these studies employed low samplings per region, except for the metropolitan region [115], which may not reflect the true levels of different bird populations. Furthermore, this scenario may indicate some degree of homogeneity for this species, with concentration peaks when exposed to point sources of contamination, such as artisanal mining at the Madeira River, and industrial/urbanized pollution sources in São Paulo, although a more detailed analysis of the area and *A. alba* populations is required for a broader understanding of these trends.

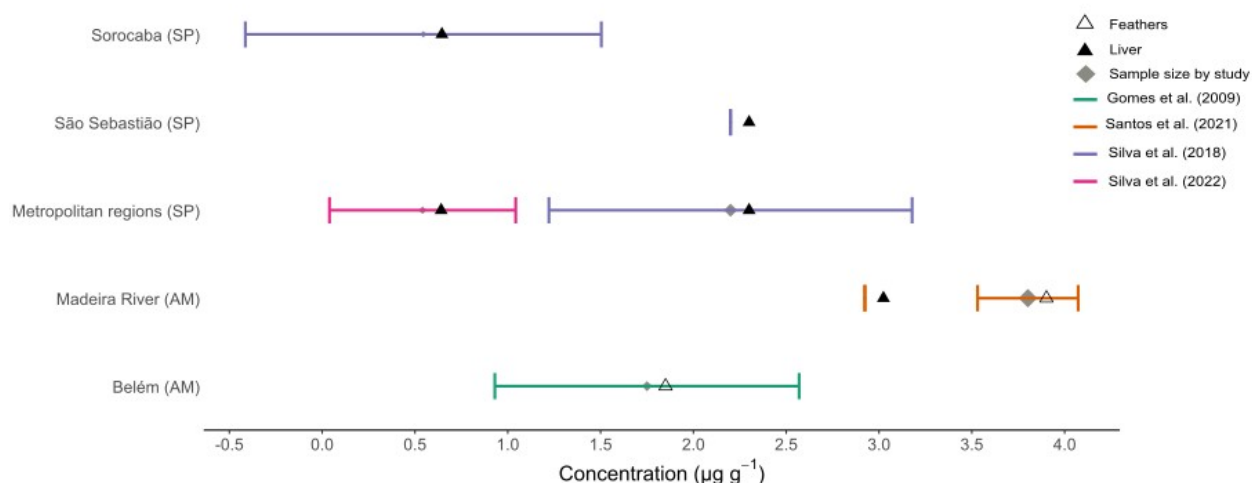


Figure 4. Mercury concentrations ($\mu\text{g g}^{-1}$) in liver and feathers of *A. alba* from different regions of Brazil. Data from Silva et al. [114], Santos et al. [51], Silva et al. [115], and Gomes et al. [87]. The error bars correspond to a Confidence Interval (CI) of 95%.

However, higher variations between studies were noted in other scenarios, even when analyzing the same matrices in the same species, probably due to intraspecific differences [64] and multiple environment contexts. When analyzing mercury in *A. alba* feathers, for example, Gomes et al. [87] reported levels of $1.75 \pm 1.29 \mu\text{g g}^{-1}$ in the urban area of Belém, in the state of Pará, northern Brazil, whereas Santos et al. [51] observed Hg concentrations of $3.85 \pm 1.51 \mu\text{g g}^{-1}$ in animals sampled from the Madeira River, 120 km from Humaitá, in the state of Amazonas, also in northern Brazil (Figure 4). The higher Hg levels observed by Santos et al. [51] are probably due to artisanal mining, which is persistent in the region [139, 140]. Both studies carried out comparable sample preparation protocols that included feather washing, so the reported Hg levels are due to internal feather deposition following metabolism, rather than external contamination.

In another study, Bighetti et al. [127] reported levels of $2.68 \pm 0.78 \mu\text{g g}^{-1}$ in *S. leucogaster* feathers from Santana Archipelago in 2018, and $4.94 \pm 1.71 \mu\text{g g}^{-1}$ in 2019 [126], while Nunes et al. [118] observed Hg levels about $0.98 \pm 1.53 \mu\text{g g}^{-1}$ for the same species from Abrolhos Archipelago and Trindade Island, two remote islands off the coast of Brazil. These reported differences might be associated with distinct geographical locations. The Abrolhos Archipelago ($17^{\circ} 58' 15.33''\text{S}$; $38^{\circ} 42' 5.99''\text{W}$) is located approximately 72 km from Caravelas (Bahia), and Trindade Island ($20^{\circ}30'55.0''\text{S}$; $29^{\circ}20'09.9''\text{W}$) is around 1,140 km distant from Vitória (Espírito Santo), while the Santana Archipelago ($22^{\circ}24'35.4''\text{S}$; $41^{\circ}42'37.2''\text{W}$) is considerably closer to the coast (~8 km from Macaé, in the state of Rio de Janeiro). Urbanization (e.g. industrial activities, metallurgy, mining activities, coal burning, and urban and industrial effluents) is a key factor in the increase of toxic elements in the biosphere, with Hg being one of the metals with predominantly anthropogenic sources [34, 39, 141, 142]. As reported by Soldatini et al. [143], *Puffinus opisthomelas* individuals that forage inshore had higher levels of Hg than those that feed offshore, explaining the correlation between Hg concentrations and coastal proximity.

Seasonal variations are also noteworthy. For example, Nunes et al. [118] compared Hg levels before (2011) and after (2019) the collapse of the Fundão Dam in southeastern Brazil in 2015. The values found by the authors suggest lower levels in 2019 (after the collapse of the Fundão Dam) in feathers of three species, namely

S. leucogaster, *P. aethereus*, and *Pterodroma arminjoniana*. As reported by Bauer et al. [72], Hg levels from 2019 to 2022 in *S. leucogaster* and *P. aethereus* feathers tend to be low ($0.94 \pm 1.31 \mu\text{g g}^{-1}$ and $0.89 \pm 1.68 \mu\text{g g}^{-1}$, respectively), except during the rainy season of 2021 when levels reached about $3 \mu\text{g g}^{-1}$ in both species. Seasonal variations may occur due to rain and consequent contaminant mobilization, as noted for fish concerning Hg [144], and transport via suspended particulate matter [145]. Given that fish are a significant part of the diet for many birds, seasonal differences may also lead to Hg variations.

In other cases, the same area may contain different Hg levels, depending on which species is being used as a bioindicator. This was reported for feathers of *S. magellanicus* ($0.78 \pm 0.44 \mu\text{g g}^{-1}$) [123], *Procellaria aequinoctialis* ($1.84 \pm 2.48 \mu\text{g g}^{-1}$) [119], and *Procellaria conspicillata* ($11.17 \pm 3.78 \mu\text{g g}^{-1}$) [119] sampled from the states of Rio Grande do Sul and Santa Catarina, in southern Brazil. The considerable differences between species may, therefore, be associated with foraging habits and trophic niche [31, 146-149], reinforcing the need for multi-species monitoring programs for a better understanding of the real contamination of a certain area.

Some discrepancies in Hg levels between matrices were observed within the same study (Figure 5). For example, similar Hg levels were observed in five analyzed species from the Madeira River (*A. alba*, *Ardea cocoi*, *Anhinga anhinga*, *Egretta thula*, and *N. brasiliense*), while pronounced difference between matrices occurred, with higher levels observed in feathers compared to liver [51]. Similarly, Costa et al. [49] reported Hg values in *Chloroceryle amazona* feathers of about $4.81 \pm 2.02 \mu\text{g g}^{-1}$, and in liver of about $2.81 \pm 1.84 \mu\text{g g}^{-1}$. However, some studies point to an inverse relation between matrices, such as Kehrig et al. [123], who observed higher Hg levels in *S. magellanicus* liver ($5.70 \pm 3.73 \mu\text{g g}^{-1}$) compared to feathers ($0.78 \pm 0.44 \mu\text{g g}^{-1}$) in the state of Rio Grande do Sul, and Oliveira et al. [50], who determined $11.04 \pm 7.85 \mu\text{g g}^{-1}$ in *Chloroceryle americana* livers and $5.97 \pm 3.17 \mu\text{g g}^{-1}$ in feathers.

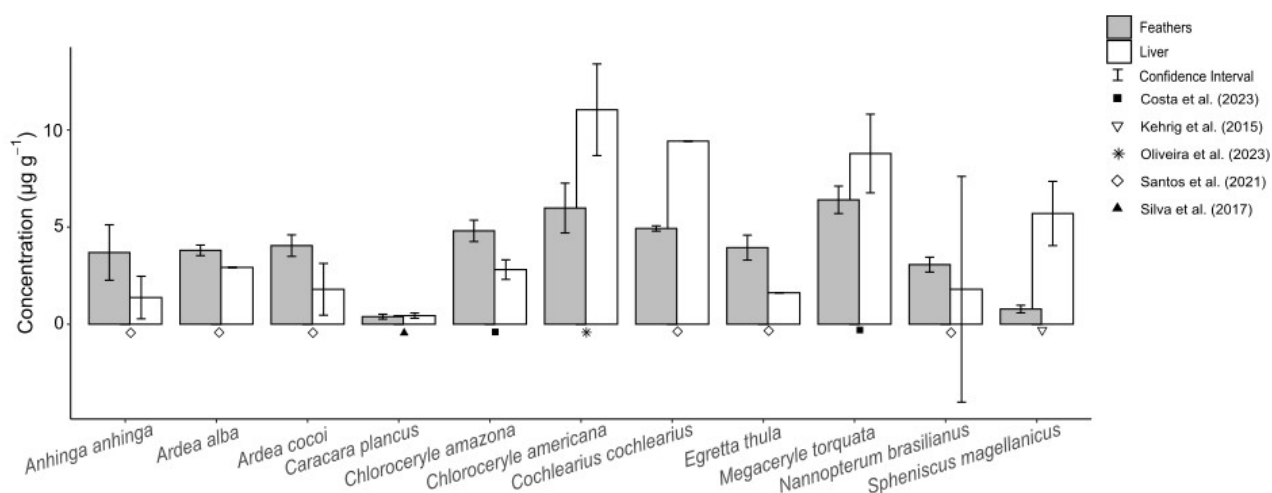


Figure 5. Comparison between mercury concentrations reported in the same studies concerning the feathers and liver of different avian species. Data from Costa et al. [49], Oliveira et al. [50], Santos et al. [51], Silva et al. [113], and Kehrig et al. [123]. The error bars correspond to a Confidence Interval (CI) of 95%.

This may be attributed to differential liver and feather deposition. Liver is a known participant in the Hg detoxification process, so higher levels are expected [39, 56, 150, 151]. However, feathers serve as a significant pathway for metal and metalloid excretion, particularly for this element [39]. Mercury levels in feathers serve as a reliable indicator of levels in blood during the feather growth period [39, 135]. Discrepancies in concentrations can, therefore, be attributed to the timing of each sampling, and feather preparation methods (e.g. feather washing for removal of external contamination).

Additionally, Hg may be present in the environment in its elemental (Hg^0), inorganic (e.g. Hg^{2+}), and organic (e.g. CH_3Hg^+) forms. Methylmercury (CH_3Hg^+) is an organometallic compound and the primary organic Hg contamination form in animals, with a high capacity for bioaccumulation and biomagnification throughout food webs [142, 152]. In this quantitative synthesis, only three studies reported methylmercury levels, as MeHg (Figure 2), highlighting the need to conduct future speciation assessments in avian species.

3.3.2 Cadmium (Cd)

Pooled estimates for Cd concentrations exhibited low variations between the two tissues ($0.26 \mu\text{g g}^{-1}$ in liver and $0.07 \mu\text{g g}^{-1}$ in feathers, Figure 6). Two among the eleven studies on liver concentrations [115, 124] exhibited CI crossing the pooled estimate line, and only one of ten studies about feathers [112] exhibited CI that crossed the pooled estimate line, indicating that the reported Cd concentrations did not vary significantly from the overall average of the studies included, due to their weights on the pooled estimate or similarity between reported concentrations. However, the remaining studies [88, 110, 112, 123, 125, 128, 136] exhibited means relatively close to the pooled estimate, with the exception of two studies that showed elevated means [72, 119].

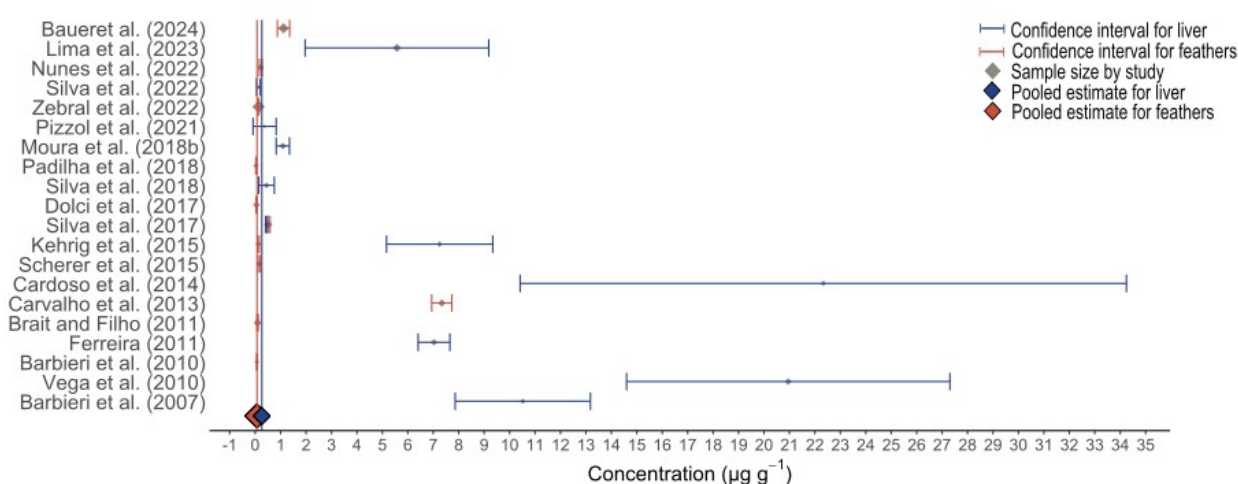


Figure 6. Forest plot for cadmium, with the pooled estimates for both liver ($0.26 \mu\text{g g}^{-1}$) and feathers ($0.07 \mu\text{g g}^{-1}$) in avian species analyzed in Brazil. The error bars correspond to a Confidence Interval (CI) of 95%.

A variety of species were analyzed concerning Cd levels in the liver. Occasionally, the values between close areas in the same species present a certain degree of uniformity that could represent similar exposure patterns. *Ardea alba* livers were analyzed in different regions of São Paulo and metropolitan cities, with similar values, the lowest being $0.13 \pm 0.07 \mu\text{g g}^{-1}$ [114], and the highest $0.48 \pm 0.84 \mu\text{g g}^{-1}$ [115].

On the other hand, the comparison of detected Cd levels in a certain region in the livers of different species was heterogeneous, as noted for the states of Rio de

Janeiro and Espirito Santo. Values as low as $0.57 \pm 0.57 \mu\text{g g}^{-1}$ in *F. magnificens*, $1.24 \pm 0.99 \mu\text{g g}^{-1}$ in *S. leucogaster*, and $1.28 \pm 0.53 \mu\text{g g}^{-1}$ in *L. dominicanus* [111], and as high as $22.33 \pm 25.46 \mu\text{g g}^{-1}$ in *Puffinus puffinus* [121], and $24.40 \pm 23.60 \mu\text{g g}^{-1}$ in *S. magellanicus* [52] were reported. As stated previously, when evaluating different species, discrepancies can arise from different trophic levels and foraging habits [148, 153].

Some of the highest Cd means in livers were detected in *S. magellanicus*, particularly in the state of Rio Grande do Sul, ranging from $7.25 \pm 4.71 \mu\text{g g}^{-1}$ [123] to $10.9 \pm 8.6 \mu\text{g g}^{-1}$ [52], and $24.4 \pm 23.6 \mu\text{g g}^{-1}$ in Rio de Janeiro [52]. This is probably due to penguins feeding on squid [130], which are known to accumulate high Cd levels [154].

Differences between tissues in one of the studies were noted, reported by Kehrig et al. [123] for *S. magellanicus* in the state of Rio Grande do Sul, where feather values were $0.13 \pm 0.07 \mu\text{g g}^{-1}$, and liver values $7.25 \pm 4.71 \mu\text{g g}^{-1}$. This is consistent with Cd levels across the feathers of Brazilian birds, contrasting with the observed variability in liver. Tissues with higher deposition of this metal tend to be the kidneys and liver [155, 156], while feather levels are usually lower [133]. It is, thus, presumed that the preferred matrix for investigating Cd in birds should be the liver, with observed variabilities indicative of regional and species-specific differences.

Two cases of particularly high Cd levels in feathers are noted. Carvalho et al. [119] reported levels of Cd in two seabirds, respectively $7.34 \pm 1.70 \mu\text{g g}^{-1}$ in *P. aequinoctialis*, and $7.33 \pm 1.57 \mu\text{g g}^{-1}$ in *P. conspicillata* in the states of Rio Grande do Sul and Santa Catarina. However, other studies conducted in the same region found lower values in different species, i.e., *S. magellanicus* from Rio Grande do Sul ($0.13 \pm 0.07 \mu\text{g g}^{-1}$) [123] and *Calidris subruficollis* ($0.15 \pm 0.10 \mu\text{g g}^{-1}$) [110], and *L. dominicanus* from Santa Catarina ($0.072 \pm 0.024 \mu\text{g g}^{-1}$) [112]. These differences may be due to local specific Cd sources, as well as foraging habits and trophic niche [148, 153].

Birds sampled at the Abrolhos Archipelago by Bauer et al. [72] from 2019 to 2022 presented Cd concentrations of $1.06 \pm 1.57 \mu\text{g g}^{-1}$ (*S. leucogaster*), and $1.17 \pm 1.86 \mu\text{g g}^{-1}$ (*P. aethereus*) in feathers. This study was conducted after the collapse of the Fundão Dam in eastern Brazil in 2015. Another study carried out in the same

area (Abrolhos Archipelago and Trindade Island) by Nunes et al. [118] reported Cd levels in feathers after the collapse of the Fundão Dam (2019) as $0.44 \pm 0.10 \mu\text{g g}^{-1}$ in *S. leucogaster*, $0.03 \pm 0.03 \mu\text{g g}^{-1}$ in *P. aethereus*, and $0.25 \pm 0.06 \mu\text{g g}^{-1}$ in *P. arminjoniana*.

In this context of the Fundão Dam collapse, animals belonging to the same species and from the same region, in a relatively close time window, and analyzed by similar sample preparation methods, exhibited discrepancies concerning Cd levels. These contrasting results may be explained by seasonal variations. Bauer et al. [72], for example, reported Cd concentration peaks in rainy periods ($3.82 \pm 2.59 \mu\text{g g}^{-1}$ in *P. aethereus*, and $3.61 \pm 2.01 \mu\text{g g}^{-1}$ in *S. leucogaster* in 2021) and lower levels during dry periods. However, low levels were detected in the rainy period of 2019 ($0.29 \pm 0.05 \mu\text{g g}^{-1}$ in *P. aethereus*, and $0.44 \pm 0.14 \mu\text{g g}^{-1}$ in *S. leucogaster*) similar to the values reported by Nunes et al. [118].

3.3.3. Lead (Pb)

The pooled estimates for Pb concentrations are remarkably similar between matrices: $0.37 \mu\text{g g}^{-1}$ for liver and $0.23 \mu\text{g g}^{-1}$ for feathers (Figure 7). Among the analyzed studies, two [120, 124] CI for liver concentrations crossed the respective pooled estimate line, whereas none of the studies on feathers did.

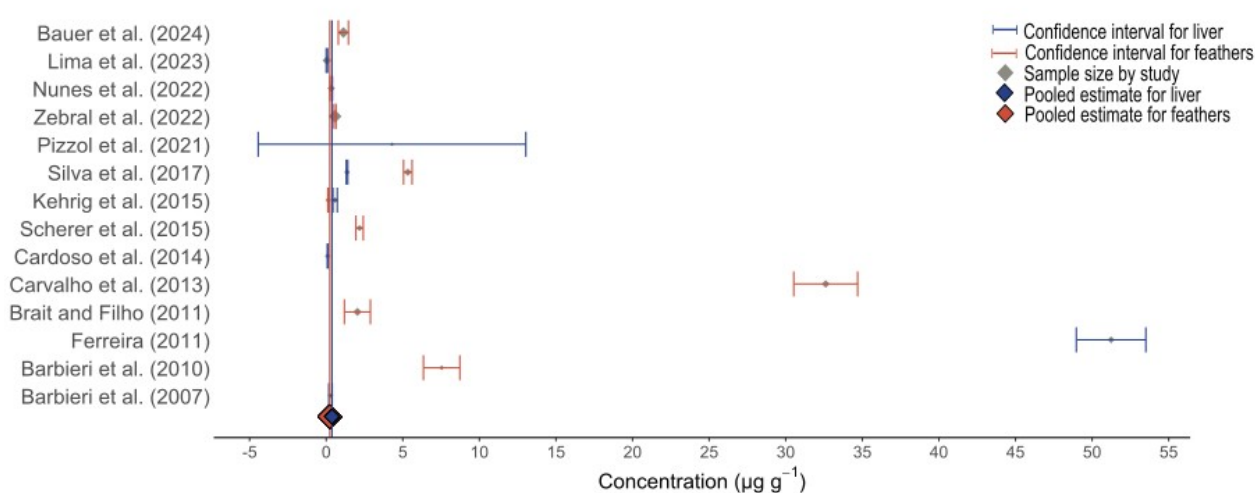


Figure 7. Forest plot for lead, with the pooled estimates for both liver ($0.37 \mu\text{g g}^{-1}$) and feathers ($0.23 \mu\text{g g}^{-1}$) in avian species analyzed in Brazil. The error bars correspond to a Confidence Interval (CI) of 95%.

With regard to liver, four of the means were found to be near the pooled estimated value, with relatively low values (e.g. [113, 121-123]). Pizzol et al. [124] reported findings that crossed the pooled estimate line, although, due to the considerable range of this CI (with an error margin of 8.74%), a lower reliability of these findings is noted. One study did not appear in the forest plot, as the authors did not report detectable levels in the liver of *S. magellanicus* from Rio de Janeiro [52]. Consistently, Kehrig et al. [123] reported low values for this species ($0.58 \pm 0.32 \mu\text{g g}^{-1}$) from the same state.

Most studies investigating Pb levels in liver reported similar low values, with some exceptions, such as Ferreira [117], who reported $51.24 \pm 7.28 \mu\text{g g}^{-1}$ in *Egretta thula* sampled from Rio de Janeiro, significantly higher than the $4.0 \mu\text{g g}^{-1}$ threshold that represents Pb liver concentrations that may cause intoxication in birds [152]. No other reports for Pb levels in this matrix and species in Brazil are available. However, when compared to another study conducted in the same region with other species, significantly different levels were noted, such as Cardoso et al. [121], who reported $0.1 \pm 0.06 \mu\text{g g}^{-1}$ in liver of *P. puffinus*. Recent studies on different matrices have reported significant metal contamination in this area (Sepetiba Bay, Rio de Janeiro) due to intensive metallurgical industry operations [157, 158]. Therefore, it seems plausible to suggest that the study by Ferreira [117] represents an example of a local source of exposure that should be further investigated.

Two studies were conducted in northeastern Brazil, reporting lower liver Pb levels in *Puffinus gravis* ($0.28 \pm 0.2 \mu\text{g g}^{-1}$) from Aracaju, in Sergipe [120] compared to levels detected in *Caracara plancus* ($1.36 \pm 0.11 \mu\text{g g}^{-1}$) sampled from Recife, Pernambuco [113]. Even though *P. gravis* is a seabird that inhabits coastal and inshore environments, which could lead to Pb exposure, reported levels in this species were lower than in *C. plancus*. *Caracara plancus* is a bird of prey on top of the food chain, so high levels of contaminants such as Pb are expected, especially due to the proximity of urbanized areas, in this case, an airport.

Concerning studies investigating Pb in feathers, four assessments reported means with only a slight discrepancy from the pooled estimate [72, 118, 123, 136]. The remaining five studies [88, 110, 112, 113, 119] presented a noteworthy distinction of the pooled estimate. Indeed, Silva et al. [113], Barbieri et al. [112], and Carvalho et

al. [119] exceeded the level of $4.0 \mu\text{g g}^{-1}$ Pb associated with sublethal damage in birds [138].

High variations were noted when assessing different species in the same area, due to different trophic levels and foraging habits [31, 148, 153]. Lower levels of $0.14 \pm 0.08 \mu\text{g g}^{-1}$ were detected in *S. magellanicus* [123], in contrast to higher levels of $2.18 \pm 0.63 \mu\text{g g}^{-1}$ observed in *C. subruficollis* [110], as well as $7.54 \pm 1.66 \mu\text{g g}^{-1}$ in *L. dominicanus* [112], and over $30 \mu\text{g g}^{-1}$ in *P. aequinoctialis* and *P. conspicillata* [119] from the states of Rio Grande do Sul and Santa Catarina.

Concerning studies in specimens from Abrolhos archipelago and Trindade island, two studies assessed Pb concentrations after the Fundão Dam collapse (2015), namely Nunes et al. [118] and Bauer et al. [72], reporting similar values for *P. aethereus* and *S. leucogaster*. These reports were typically below $1 \mu\text{g g}^{-1}$, with some seasonal variability exceptions reported in the rainy season of 2021 (4.63 ± 4.33 and $4.18 \pm 2.85 \mu\text{g g}^{-1}$ for the two species respectively) [72].

3.3.4. Essential metals (Cr, Cu and Zn)

Metals such as Cu, Cr, and Zn are essential for living organisms ([39, 142, 159-162]. These elements compose several biological processes, including protein synthesis and metabolism, enzymatic regulation, carbohydrate metabolism, and antioxidant system regulation [142, 159-161]. The results of the present quantitative synthesis, concerning these three metals in liver and feathers in Brazilian birds, are presented below.

The Cr pooled estimate for liver was $0.82 \mu\text{g g}^{-1}$, and for feathers, $2.72 \mu\text{g g}^{-1}$ (Figure 8, A). None of the retrieved papers regarding Cr levels in liver, and the one concerning feathers [118], indicated a CI crossing their respective pooled estimate line. Lima et al. [122] was the only liver assessments with values below the pooled estimate.

The liver means were relatively close to each other, as noted for specimens from Rio de Janeiro and Espírito Santo states from four different species. Levels ranged from $1.16 \pm 0.46 \mu\text{g g}^{-1}$ in *S. leucogaster* [111], $2.02 \pm 0.30 \mu\text{g g}^{-1}$ in *F. magnificens* [111], $2.58 \pm 0.70 \mu\text{g g}^{-1}$ in *E. thula* [117], and $2.74 \pm 0.41 \mu\text{g g}^{-1}$ in *L. dominicanus* [111]. Pizzol et al. [124] reported a high CI variability (ranging from 4.82

to 23.05) in Cr concentrations in liver, potentially due to the number of studied species. The lowest Cr level reported by these authors was $3.78 \mu\text{g g}^{-1}$, and the highest $22.10 \pm 0.94 \mu\text{g g}^{-1}$. However, the sampling number of each species was low (one individual for *Megascops* spp., *Pulsatrix koeniswaldiana*, and *Asio stygius*, and two for *Tyto furcata*), hindering assessments concerning the real Cr pattern in these species in the sampled area.

As for feathers, three studies present values above the pooled estimate [72, 112, 113], and three below [88, 136, 163], indicating higher Cr variability in feathers than in liver. It is possible to notice this in some studies of the south region: levels of $4.67 \pm 1.26 \mu\text{g g}^{-1}$ were reported in *L. dominicanus* of Santa Catarina [112], in contrast with Scherer et al. [110] who did not detect this metal in *C. subruficollis* feathers from Rio Grande do Sul.

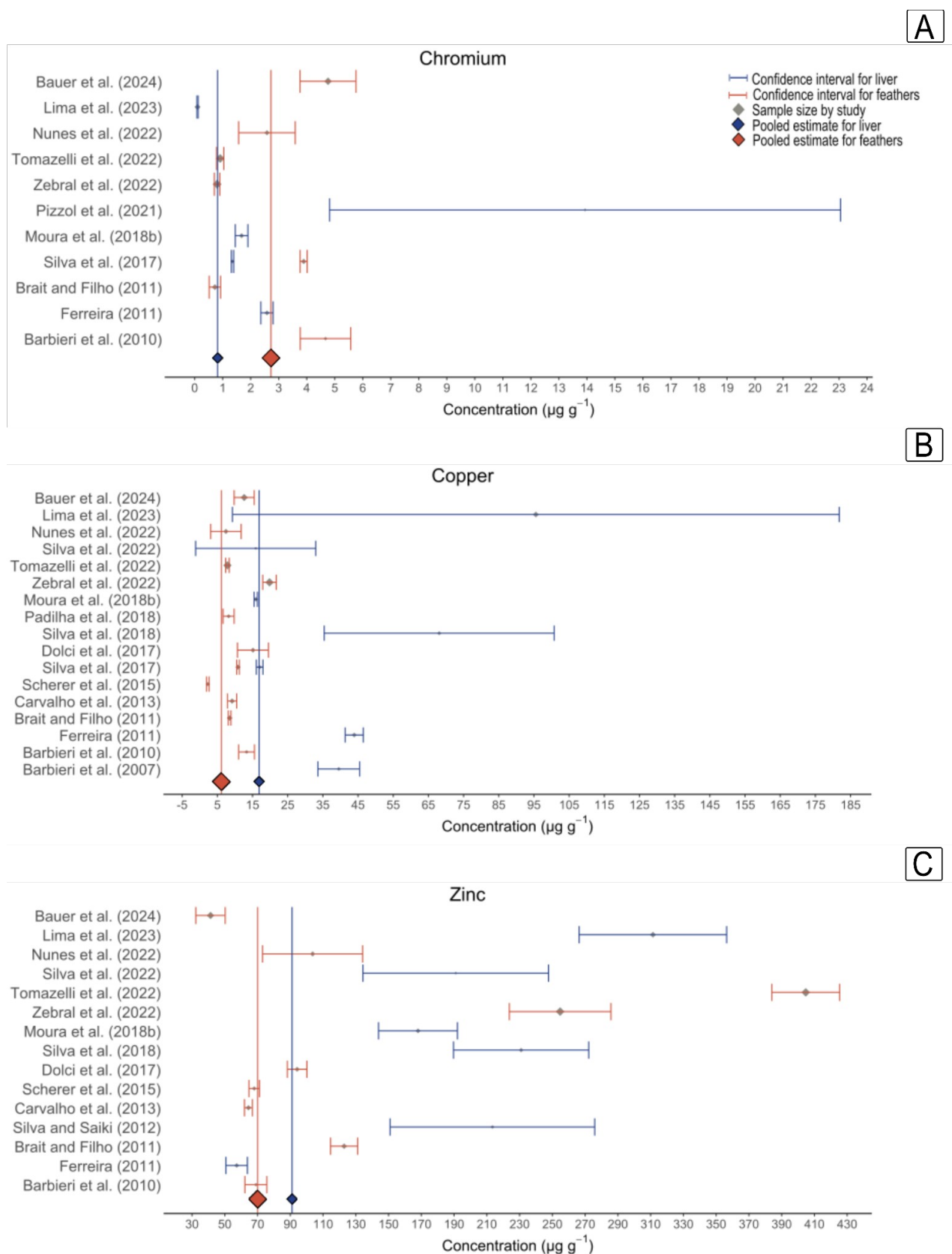


Figure 8. Forest plot for essential metals in avian species analyzed in Brazil. **(A)** Chromium, with the pooled estimates for liver ($0.82 \mu\text{g g}^{-1}$) and feathers ($2.72 \mu\text{g g}^{-1}$).

(B) Copper, with the pooled estimates for liver ($16.87 \mu\text{g g}^{-1}$) and feathers ($6.15 \mu\text{g g}^{-1}$). **(C)** Zinc, with the pooled estimates for liver ($91.07 \mu\text{g g}^{-1}$) and feathers ($70.05 \mu\text{g g}^{-1}$). The error bars correspond to a Confidence Interval (CI) of 95%.

Regarding Cu, the pooled estimate for liver was $16.87 \mu\text{g g}^{-1}$ and for feathers was $6.15 \mu\text{g g}^{-1}$ (Figure 8, B). Three studies examining Cu concentrations in liver indicated a CI crossing the pooled estimate line [113, 114, 122], and one reported means below but very close to this value [111]. Conversely, only one study on feathers exhibited a CI crossing this line [118], and another reported had means below this value [110].

Although Lima et al. [122] reported values over the pooled estimate line for liver, that study exhibited an 86.25% error margin, with the CI ranging from 9.29 to 181.78. In this particular case, the authors analyzed birds from the states of Santa Catarina, Paraná, São Paulo, and Rio de Janeiro, hindering accurate concentration assessments per region. The observed high variability could also be attributed to the high Cu level variations between species, i.e., $253.53 \pm 942 \mu\text{g g}^{-1}$ in *Thalassarche melanophris*, $17.96 \pm 7.21 \mu\text{g g}^{-1}$ in *Thalassarche chlororhynchos*, and $59.73 \pm 64.6 \mu\text{g g}^{-1}$ in *N. brasilianum*.

Copper values in liver differed when analyzing different species from the same region, as in the case of the Brazilian northeast, with reports of $39.51 \pm 10.73 \mu\text{g g}^{-1}$ in *P. gravis* from Sergipe [120], and $17.03 \pm 2.07 \mu\text{g g}^{-1}$ in *C. plancus* from Pernambuco [113]. However, even on a small spatial scale and using only *A. alba* as indicator, Silva et al. [115] reported $77.0 \pm 68.6 \mu\text{g g}^{-1}$ in metropolitan São Paulo regions, contrasting with other reports from the same area ($15.9 \pm 1.9 \mu\text{g g}^{-1}$ [114]) and in nearby cities ($23.40 \pm 0.04 \mu\text{g g}^{-1}$ in Sorocaba, in the state of São Paulo [115], as well as values below the limit of detection ($8.6 \mu\text{g g}^{-1}$) in São Sebastião, also in the state of São Paulo [115]. The only case of similar levels was observed in three seabird species in Rio de Janeiro and Espírito Santo: Moura et al. [111] reported levels of $16.49 \pm 0.29 \mu\text{g g}^{-1}$ in the liver of *S. leucogaster*, $17.61 \pm 0.24 \mu\text{g g}^{-1}$ in *L. dominicanus*, and $13.23 \pm 0.20 \mu\text{g g}^{-1}$ in *F. magnificens*.

Similarly, *S. leucogaster* feathers showed different Cu values in distinct regions, such as $8.20 \pm 3.17 \mu\text{g g}^{-1}$ at the Cagarras Archipelago [125], and $15.12 \pm$

15.70 $\mu\text{g g}^{-1}$ in the state of Paraná [128]. Additionally, four species of seabirds analyzed in the south region (Rio Grande do Sul and Santa Catarina) varied widely, *i.e.*, $13.30 \pm 3.15 \mu\text{g g}^{-1}$ in *L. dominicanus* [112], $10.74 \pm 5.56 \mu\text{g g}^{-1}$ in *P. aequinoctialis* [119], $7.97 \pm 5.05 \mu\text{g g}^{-1}$ in *P. conspicillata* [119], and $2.29 \pm 0.95 \mu\text{g g}^{-1}$ in *C. subruficollis* [110].

Concerning Zn, the pooled estimate for liver was $91.07 \mu\text{g g}^{-1}$, and for feathers $70.05 \mu\text{g g}^{-1}$ (Figure 8, C). None of the six studies that reported Zn concentrations in the liver yielded a CI that crossed the pooled estimate line, and only one study was below this value [117]. Two of nine studies conducted on feathers [110, 112] yielded a CI over the pooled estimate line, and two exhibited mean values below [72, 119].

Some variations referring to Zn in liver were noted for specimens sampled from Rio de Janeiro and Espírito Santo. These studies reported levels in different species, such as *E. thula* ($57.26 \pm 21.1 \mu\text{g g}^{-1}$) [117], *S. leucogaster* ($96.88 \pm 0.37 \mu\text{g g}^{-1}$) [111], *L. dominicanus* ($253.43 \pm 0.19 \mu\text{g g}^{-1}$) [111], and *F. magnificens* ($266.06 \pm 0.32 \mu\text{g g}^{-1}$) [111]. Variations between species are expected, due to different food preferences and trophic niches [164].

Only one species was assessed in the same region, *A. alba* in São Paulo state (metropolitan region and cities). Similar levels were reported for the liver of this species, *i.e.*, $191 \pm 54 \mu\text{g g}^{-1}$ in São Paulo [114], $135.9 \pm 26.8 \mu\text{g g}^{-1}$ in the city of Sorocaba [115], and $186.7 \pm 6.8 \mu\text{g g}^{-1}$ in the city of São Sebastião [115]. Slightly higher values were found in specimens from the city of São Paulo ($213.4 \pm 93.0 \mu\text{g g}^{-1}$) [116], and the metropolitan region of this state ($243.6 \pm 112.9 \mu\text{g g}^{-1}$) [115]. Similar concentrations in a given region may potentially contribute to monitoring strategies that can help the perception of possible Zn deficiencies, or even damage caused by Zn exposure.

The two studies on feathers reporting high means [136, 163] were conducted without species differentiation (use of “Composite Samples”), hindering assessments. Concentrations in feathers, in general, were similar between different species in studies conducted in the same region, *i.e.*, in southern Brazil (Paraná, Santa Catarina, and Rio Grande do Sul), concentrations of $94.16 \pm 21.23 \mu\text{g g}^{-1}$ were found in *S. leucogaster* [128], $67.48 \pm 11.64 \mu\text{g g}^{-1}$ in *P. aequinoctialis* [119], $62.05 \pm 7.58 \mu\text{g g}^{-1}$

g⁻¹ in *P. conspicillata* [119], and $67.97 \pm 8.45 \mu\text{g g}^{-1}$ in *C. subruficollis* [110]. This similarity can be due to the typical Zn deposition in feathers.

Essential metal levels, such as Cu, Cr and Zn, are expected to exceed those of toxic metals (Cd, Hg, and Pb) [165], which was corroborated by the data reported herein in this review. Nevertheless, high concentrations of essential elements also have the potential to cause physiological and metabolic damage, usually associated with increased oxidative stress [160, 166]. It is thus essential to establish safe levels for these elements in avian tissues to verify potential toxicity [34, 142, 152, 167].

3.4. Other metals and metalloids of interest

In addition to the six most studied elements in birds in Brazil, other metals and metalloids are also significant due to the risks they pose to birds and other animals.

Arsenic was assessed in birds in seven published studies in Brazil across a range of matrices, including blood, feathers, liver, muscle, and eggshells [68, 72, 111, 118, 122, 128, 136]. This element is a metalloid that undergoes intense anthropogenic deposition in the environment through mining, industrial activities, and fossil fuel burning [34, 97, 141, 142]. It represents a significant risk, as it is carcinogenic, also capable of disrupting the antioxidant system, and causing damage to cellular membranes and DNA [34, 39, 168, 169] and may lead to kidney and liver toxicity, neurological impairment, and hematopoietic system suppression [34, 168, 169], as observed in birds and other vertebrates [34, 169, 170]. Additionally, there is evidence that this metalloid may contribute to bird eggshell thickening [34, 39].

There is a relatively limited body of research examining Rb and Ti in biota, and particularly in birds. In this sense, three studies investigated Rb [114-116], while no studies on Ti were retrieved.

Rubidium may induce nephrotoxicity in birds [168], although its potential bioaccumulation throughout food webs is still unknown [141]. However, Campbell et al. [171] suggest that Rb can increase in fishes that occupy higher trophic levels. Moreover, Hauser-Davis et al. [172] investigated Rb in muscle and liver samples of a vulnerable coastal shark (*Rhizoprionodon porosus*) in Brazil, detecting its presence in all samples. Thus, as a consequence of occupying different trophic levels, some bird species may be key to a better understanding of Rb bioaccumulation.

Studies investigating Ti in other animals in Brazil, such as crustaceans [173], have indicated the consistent detection of this element in marine environments, thus comprising a concern mainly for seabirds. Moreover, titanium dioxide nanoparticles ($\text{TiO}_2\text{-NP}$) comprise an emergent concern in terms of environmental contamination, able to accumulate in exposed organisms and cause deleterious effects, including genotoxicity [174-176]. In addition to this direct effect, $\text{TiO}_2\text{-NP}$ can also act as carriers for other metals and metalloids such as As, Cd, Cu, Pb and Zn, due to their high absorption capability [177-180].

Selenium (Se), on the other hand, is an essential element responsible for a range of metabolic processes used to guarantee homeostasis, including antioxidant effects [34, 168]. This element plays an important role in detoxification mechanisms, especially against Hg (and methylmercury), Cd, and Pb [34, 70, 123]. Selenium values were reported in ten studies in this quantitative synthesis (Figure 2). Molar ratio Se:Hg calculations, a tool routinely employed to detect Hg toxicity in fish, but not in birds, therefore, are required to assess potentially protective effects against Hg toxicity in avian species. Molar ratios concerning other toxic elements should also be employed.

4. Conclusions

This quantitative synthesis provides a comprehensive estimate of the typical concentrations of the most studied metals (Hg, Cd, Pb, Zn, Cu and Cr) found in the most investigated bird tissues (feathers and liver) in Brazil. Future studies should continue to prioritize these metals due to their high toxicity and known impacts on bird health and ecosystems. Furthermore, different contaminants, such as As, Rb, and Ti, are also worth investigating, alongside essential elements that may interact with contaminants, such as Se.

The three most commonly employed analytical techniques for measuring metals in bird tissues in Brazil were noted as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Cold Vapour Atomic Absorption Spectroscopy (CVAAS), and Atomic Absorption Spectroscopy (AAS). These techniques have been proven effective in this sense, and their consistent use facilitates reliable comparisons across studies.

While *A. alba*, *S. leucogaster*, *L. dominicanus*, and *S. magellanicus* were the most frequently studied species, expanding research to include a broader range of species is important, in order to obtain a more comprehensive understanding of metal and metalloid contamination across diverse habitats and ecological niches, improving conservation and management strategies.

The two most studied bird tissues in Brazil were feathers and liver, with certain similarities observed between matrices. This indicates the potential for the use of feathers as a less invasive matrix in biomonitoring efforts. Further research is, however, required to gain a deeper understanding of metal and metalloid deposition in feathers and ratios between internal organs, such as the liver.

Certain study limitations should be noted. For example, interspecific variation and geographical differences may have introduced potential result variability, and the current literature does not yet provide sufficient data to enable a quantitative synthesis that distinguishes metal and metalloid patterns between species and geographical locations. As a result, data representativeness and comparability across regions is hindered.

A range of variations between the mean metal and metalloid concentrations was observed, including intraspecific, regional, and environmental differences, as well as different sampling and analytical methodologies. This highlights the importance of standardized protocols to enhance the reliability and comparability of future searches.

Finally, to better understand contamination trends, long-term monitoring programs are recommended, which allow for tracking changes in contamination levels and the effectiveness of mitigation measures.

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Credit Author Statement

LSP: Investigation; Data curation; Formal analysis; Software; Writing- Original draft preparation; Writing- Reviewing and Editing. **MSTAL:** Investigation; Data curation; Formal analysis; Software; Writing- Original draft preparation; Writing- Reviewing and Editing. **SS:** Supervision; Conceptualization; Methodology; Funding acquisition; Project administration; Resources; Writing- Original draft preparation; Writing- Reviewing and Editing. **RAHD:** Supervision; Conceptualization; Methodology; Funding acquisition; Project administration; Resources; Writing- Original draft preparation; Writing- Reviewing and Editing.

Declaration of Generative AI and AI-assisted technologies in the writing process

The ChatGPT software was used for clearer grammar and syntax. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the reported contents.

Ethics approval and consent to participate

This study is a literature review and did not involve the use of live animals or human participants. Only previously published secondary data were analyzed. Therefore, ethical approval and consent to participate were not required. All reviewed studies referenced herein were assumed to have been conducted in accordance with ethical standards applicable at the time of their publication.

Declaration of interests

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

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Supplementary Materials

1. Material and methods

1.1. Pooled estimate, and confidence interval

For the calculations of the weighted mean (e.g. pooled estimate), the recommendation by Harrer et al. [1] was followed. The *weights* (w), influenced by the *standard deviation* (sd) of each study, were considered (**Equation 1**).

$$w = \frac{1}{sd^2} \quad (1)$$

The weighted mean (M), variance (S), and standard deviation (SD) were calculated using the weights (w) and means (m) of each study, and the **(Equation 2)**.

$$M = \frac{\sum mw}{\sum w}$$

$$S = \frac{1}{\sum w}$$

$$SD = \sqrt{S}$$

(2)

1.2. Mean and standard deviation (sd) estimation

When the studies reported multiple means and sd for different groups (e.g. females/males), an overall unified mean was calculated as a weighted average of the group means, considering the sample sizes [2]. When the sd was not provided in the studies, this parameter was estimated from the *range* (e.g. difference between minimum and maximum values) as suggested by Hozo et al. [3] **(Equation 3)**.

$$sd = \frac{range}{4}$$

(3)

1.3. Conversions of wet weight to dry weight

For the conversion of the concentrations in wet weight (C_{ww}) to the concentrations in dry weight (C_{dw}), 70% of water content in the liver was assumed. The conversion factor (CF) was taken into account, and **Equation 4** represents the method utilized [4].

$$CF = 1 - watercontent$$

$$C_{ww} = C_{dw} \times CF$$

(4)

2. Results

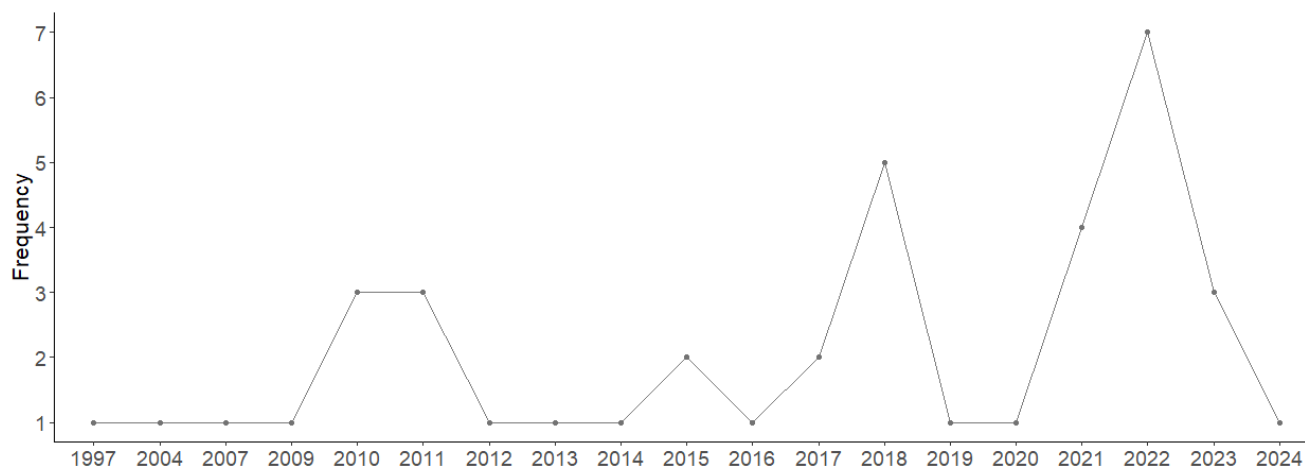


Figure S1: Number of studies (Frequency) published in Brazil, per year (1997 to 2024), investigating metals, and metalloids in avian species.

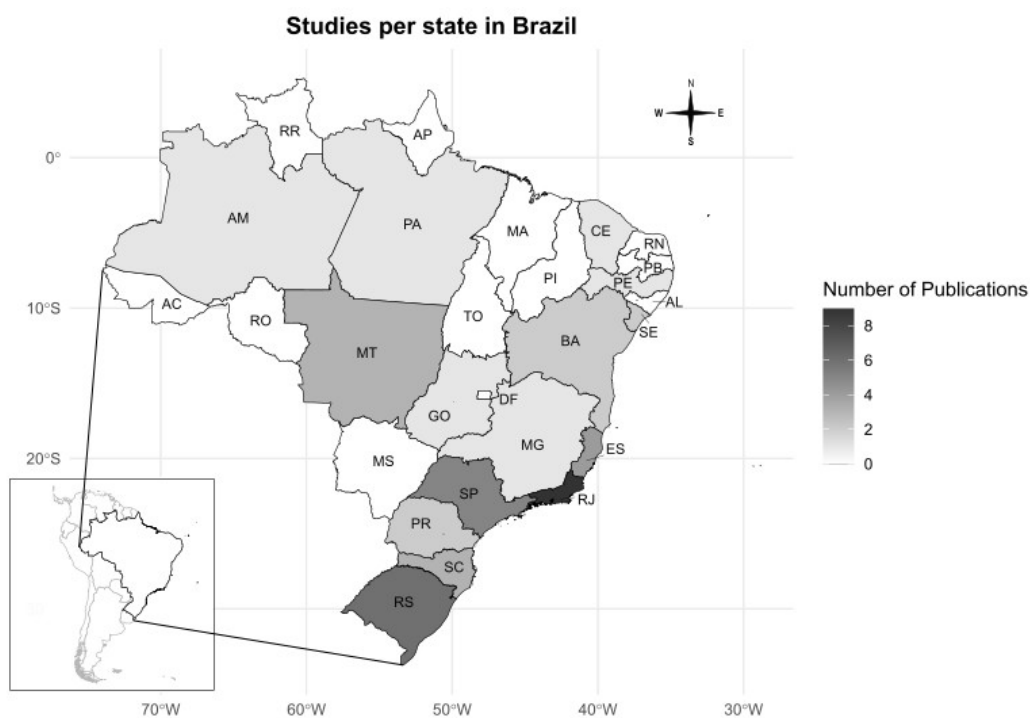


Figure S2: Number of studies conducted per state investigating metals, and metalloids in avian species published in Brazil. The map illustrates the distribution with color gradients representing the volume of publications. Darker shades indicate a higher number of studies, while lighter shades represent fewer studies.

Supplementary References

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