

INDICADORES BIOGEOQUÍMICOS E ECOTOXICOLÓGICOS
APLICADOS À DIFERENCIAÇÃO DE DUAS ÁREAS ESTUARINAS
COM DISTINTAS PRESSÕES ANTRÓPICAS (RIO PARAÍBA DO SUL –
RJ E RIO CAETÉ – PA)

ÉCHILY SARTORI

UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY
RIBEIRO – UENF

CAMPOS DOS GOYTACAZES – RJ

JUNHO – 2026

INDICADORES BIOGEOQUÍMICOS E ECOTOXICOLÓGICOS
APLICADOS À DIFERENCIAÇÃO DE DUAS ÁREAS ESTUARINAS
COM DISTINTAS PRESSÕES ANTRÓPICAS (RIO PARAÍBA DO SUL –
RJ E RIO CAETÉ – PA)

ÉCHILY SARTORI

Tese apresentada ao Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para a obtenção do título de Doutora em Ecologia e Recursos Naturais.

Orientador: Prof. Dr. Carlos Eduardo de Rezende

Coorientadora: Prof.^a Dr.^a Cristiane dos Santos Vergilio

CAMPOS DOS GOYTACAZES – RJ

JUNHO – 2026

FICHA CATALOGRÁFICA

UENF - Bibliotecas

Elaborada com os dados fornecidos pela autora.

S251

Sartori, Échily.

INDICADORES BIOGEOQUÍMICOS E ECOTOXICOLÓGICOS APLICADOS À DIFERENCIAÇÃO DE DUAS ÁREAS ESTUARINAS COM DISTINTAS PRESSÕES ANTRÓPICAS (RIO PARAÍBA DO SUL - RJ E RIO CAETÉ - PA) / Échily Sartori. - Campos dos Goytacazes, RJ, 2026.

147 f. : il.

Inclui bibliografia.

Tese (Doutorado em Ecologia e Recursos Naturais) - Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Biociências e Biotecnologia, 2026.

Orientador: Carlos Eduardo de Rezende.

Coorientadora: Cristiane dos Santos Vergilio.

1. contaminação ambiental. 2. ecotoxicologia. 3. metais. 4. POPs. I. Universidade Estadual do Norte Fluminense Darcy Ribeiro. II. Título.

CDD - 577

INDICADORES BIOGEOQUÍMICOS E ECOTOXICOLÓGICOS
APLICADOS À DIFERENCIAÇÃO DE DUAS ÁREAS ESTUARINAS
COM DISTINTAS PRESSÕES ANTRÓPICAS (RIO PARAÍBA DO SUL –
RJ E RIO CAETÉ – PA)

ÉCHILY SARTORI

Tese apresentada ao Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para a obtenção do título de Doutora em Ecologia e Recursos Naturais.

Aprovada em 26 de junho de 2026.

Comissão Examinadora:

Documento assinado digitalmente
 **NILS EDVIN ASP NETO**
Data: 10/07/2026 07:16:24-0300
Verifique em <https://validar.iti.gov.br>

Prof. Dr. Nils Edvin Asp Neto - UFPA

Documento assinado digitalmente
 **MARIANNA BASSO JORGE**
Data: 08/07/2026 16:07:09-0300
Verifique em <https://validar.iti.gov.br>

Prof.^a Dr.^a Mariana Basso Jorge - UFMA

Documento assinado digitalmente
 **MAURICIO MUSSI MOLISANI**
Data: 10/07/2026 11:58:10-0300
Verifique em <https://validar.iti.gov.br>

Prof. Dr. Maurício Mussi Molisani - UFRJ

Documento assinado digitalmente
 **CRISTIANE DOS SANTOS VERGILIO**
Data: 09/07/2026 18:06:08-0300
Verifique em <https://validar.iti.gov.br>

Prof.^a Dr.^a Cristiane dos Santos Vergilio (Coorientadora) - UFES

Documento assinado digitalmente
 **CARLOS EDUARDO DE REZENDE**
Data: 11/08/2026 10:52:04-0300
Verifique em <https://validar.iti.gov.br>

Prof. Dr. Carlos Eduardo de Rezende (Orientador) - UENF

Dedico essa tese aos mestres, amigos e principalmente à minha família que, direta ou indiretamente, estiveram presentes nessa caminhada.

Agradecimentos

Mais de quatro anos depois, me encontro aqui olhando para essa parte sem saber por onde começar. Foram tantos aprendizados, experiências acadêmicas, profissionais e pessoais compartilhadas com tantas pessoas, que, desde já, ficam meus sinceros agradecimentos a todos que, durante esses quatro anos, passaram pela minha vida e trouxeram suas contribuições nos diversos âmbitos dela.

Primeiramente, agradeço aos meus pais, Aloísio e Rosana Sartori, que, desde 2014, quando saí de casa pela primeira vez para cursar Ciências Biológicas em Alegre, na Universidade Federal do Espírito Santo, não mediram esforços para que eu pudesse ter todas as condições necessárias e focar na minha formação. E assim seguiu quando me mudei para Campos para me tornar aluna de mestrado, permanecendo até a finalização de mais uma etapa da minha formação durante este doutorado. Meu muito obrigado por todo apoio e suporte, muitos seriam imensamente felizes em ter vocês como pais.

Em especial, um agradecimento ao meu orientador Carlão (Carlos Eduardo de Rezende), e a minha co-orientadora Cris (Cristiane dos Santos Vergílio). Me lembro da primeira vez que conheci a Cris e me interessei por seu trabalho, e assim, lá se vão 10 anos aprendendo cada dia um pouco mais com a incrível pessoa e profissional que ela é. Agradeço por ter acreditado naquela “adolescente” e a indicado ao mestrado na UENF. E assim inicia minha jornada como aluna do Carlão, e lá se vão quase 7 anos sob sua orientação. Gostaria de deixar aqui registrado uma frase que Carlão um dia me disse quando em momentos de estresse ele conseguiu me acalmar dizendo: *“É natural que, em alguns momentos, o cansaço e a preocupação façam com que as situações pareçam mais difíceis ou negativas do que realmente são. Ainda assim, é importante lembrar de toda a trajetória já construída, do esforço dedicado até aqui e da capacidade que você tem para concluir essa etapa”*. Meu muito obrigada Carlão. Foram anos de muitos aprendizados, de muito suporte, incentivo e o mais importante, nunca descreditando do meu potencial e da profissional que serei. Me inspiro em vocês e no trabalho de vocês!

Agradeço também a minha namorada Isabela Alves, que sempre me apoiou, e nunca deixou de acreditar em mim, sempre me incentivando a fazer e ser melhor, não me deixando cair e surtar no final dessa tese. Não tenho palavras para te agradecer!

Agradeço também aos meus irmãos Juninho e Lara Sartori, pelo apoio e por saber que sempre poderei contar com eles.

Agradeço aos amigos que sempre estiveram presentes e contribuíram de alguma forma na minha formação, e na minha vida. Em especial, fica aqui muito obrigada ao Diego Lacerda e a Tassiana Serafim, que a todo o momento estiveram presentes para compartilhar da vida e dos estresses acadêmicos. Agradeço também aos amigos daquele grupo da vaquinha, dos amigos dos 4 cantos da zona da mata mineira, onde boas risadas e bons momentos foram tidos.

Aos amigos e técnicos do laboratório e os amigos que o laboratório me trouxe, meu muito obrigada pelo apoio a amizade construída ao longo desses muitos anos. Aos meus alunos de iniciação científica por terem acreditado em mim para sua orientação. Meu muito obrigada, vocês foram importantes para essa formação!

A Universidade Estadual do Norte Fluminense Darcy Ribeiro, ao Laboratório de Ciências Ambientais, e ao programa de Pós-Graduação em Ecologia e Recursos Naturais, pela estrutura, pelo espaço físico, pelos equipamentos utilizados durante esta etapa e principalmente pela obtenção do título de Doutora. Agradeço também às universidades parceiras, onde parte dessa pesquisa foi feita. A Universidade Federal do Pará, pelo suporte e logística na hora do campo e coleta das amostras. A Universidade Federal do Espírito Santo, em especial ao Laboratório de Ecotoxicologia (LABTOX), por toda parte de ensaios ecotoxicológicos, e a Constructor University, na Alemanha, por ter me recebido para realização de parte do meu doutorado. Em especial a professora Andrea Koschinsky pela supervisão e apoio.

À CAPES pela concessão da bolsa de doutorado e da bolsa do programa de doutorado sanduíche. Ao CNPq pelo financiamento do Instituto Nacional de Ciência e Tecnologia de Transferência de Materiais Continente-Oceano (INCT-TMC Ocean; Processo nº 405765/2022-3), que forneceu suporte para o desenvolvimento desta pesquisa.

A banca examinadora pelo aceite do convite e considerações feitas do trabalho.

E finalmente a todos aqueles que contribuíram direta ou indiretamente para a conclusão dessa etapa. Com certeza vocês foram importantes e levarei um pouco de cada para profissional que almejo ser. Meu muito obrigada, esse título também é de vocês.

“One day, in retrospect, the years of struggle will strike you as the most beautiful.”

- Sigmund Freud

Sumário

LISTA DE ABREVIATURAS	xii
LISTA DE TABELAS	xv
LISTA DE FIGURAS	xvi
RESUMO	xx
ABSTRACT	xxi
ESTRUTURA DA TESE	xxii
INTRODUÇÃO GERAL	1
CAPÍTULO 1 - Land-use impacts on Amazon and Atlantic Rainforest coastal ecosystems revealed by multiple biogeochemical markers	8
Abstract	8
Introduction	9
Material and Methods	11
<i>Amazon Rainforest hydrographic basin</i>	11
<i>Atlantic Rainforest hydrographic basin</i>	12
<i>Field sampling</i>	12
<i>Laboratory Analysis</i>	13
<i>Land Use Analysis</i>	14
<i>Statistical Analysis</i>	14
Results	16
<i>Land-use change between 1985 and 2024</i>	16
<i>Elemental and isotopic composition of organic matter</i>	18
<i>Organic pollutants distribution and sources</i>	22
Discussion	26
<i>Deforestation legacies and urban pressures</i>	26
<i>Stable isotopes reflect land-use legacies</i>	27
<i>Fingerprints of anthropogenic land-use through POPs</i>	30
Conclusion	34
CRedit authorship contribution statement	35
Acknowledgments	35
Funding statement	35
Declaration of Interest Statement	36

Declaration of Generative AI and AI-assisted technologies in the writing process	36
Data availability	36
References	36
Supplementary Material	47
CAPÍTULO 2 - Drivers of sediment toxicity in tropical estuaries through contaminant mixture modeling	49
Abstract	49
Introduction	50
Material and Methods	53
<i>Study Area and Sampling</i>	53
<i>Sediment Characterization</i>	54
<i>Total Metal Determination</i>	54
<i>POPs Determination</i>	55
<i>Quality Control and Assurance</i>	55
<i>Toxicity Test</i>	56
<i>Statistical Analysis</i>	57
Results	59
<i>Sediment Contamination Across Tropical Estuarine Systems</i>	59
<i>Reproductive Toxicity of Sediment-exposed Copepods</i>	62
<i>Contaminant Mixture Effects on Reproductive Output</i>	63
<i>Linking Contaminant Mixture to Reproductive Toxicity</i>	66
Discussion	66
Conclusion	72
CRedit authorship contribution statement	72
Acknowledgments	73
Funding statement	73
Declaration of interest statement	73
Declaration of generative AI and AI-assisted technologies in the writing process	74
Data availability	74
References	74
Supplementary material	82

CAPÍTULO 3 - Spatiotemporal dynamics of physical, chemical and biological water quality parameters in the Paraíba do Sul River Basin: A decade of data analysis	84
Abstract	85
Introduction	85
Material e Methods	87
<i>Study area</i>	<i>87</i>
<i>Hydrological monitoring: Programs and data selection</i>	<i>89</i>
<i>Data processing</i>	<i>90</i>
<i>Data analysis.....</i>	<i>92</i>
Results.....	94
<i>Overall water quality status</i>	<i>94</i>
<i>Temporal trends: Interannual annual and seasonal variations</i>	<i>99</i>
<i>Finer scale spatial patterns</i>	<i>102</i>
<i>Associations between variables and PSR basin sections</i>	<i>104</i>
Discussion.....	106
Conclusion	109
Statements and Declarations.....	110
<i>Conflict of Interest</i>	<i>110</i>
<i>Ethical Approval</i>	<i>110</i>
<i>Informed Consent.....</i>	<i>110</i>
<i>Clinical Trial Registration.....</i>	<i>111</i>
<i>Data Availability.....</i>	<i>111</i>
Funding.....	111
Acknowledgments	111
Author Contributions.....	111
References	112
Supplementary Material	117
CONSIDERAÇÕES FINAIS	118
REFERENCIAS BIBLIOGRÁFICAS	120

LISTA DE ABREVIATURAS

AGEVAP – Agência das Bacias Hidrográficas dos Rios Vparaí e Pomba

AIC – Akaike Information Criterion

ANOVA – Analysis of Variance

ART – Aligned Rank Transform

BC – Black Carbon

BOD – Biological Oxygen Demand

C:N_a – Carbon-to-Nitrogen ratio

CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CBH-BPSI – Comitê de Bacia Hidrográfica do Baixo Paraíba do Sul e Itabapoana

CETESB – Companhia Ambiental do Estado de São Paulo

CONAMA – Conselho Nacional do Meio Ambiente

CR – Caeté River

CTC – Capacidade de Troca Catiônica

DDD – Dichlorodiphenyldichloroethane

DDE – Dichlorodiphenyldichloroethylene

DDT – Dichlorodiphenyltrichloroethane e Dicloro-Difenil-Tricloroetano

DO – Dissolved Oxygen

FAPERJ – Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro

FAPES – Fundação de Amparo à Pesquisa do Espírito Santo

GC – Gas Chromatography

HCH – Hexachlorocyclohexane

HMW – High Molecular Weight

HPA – Hidrocarboneto Policíclico Aromático

HUWRM – Hydrological Units for Water Resources Management

HWK – Hanse-Wissenschaftskolleg – Institute for Advanced Study

IAEA – International Atomic Energy Agency

IGAM – Instituto Mineiro de Gestão das Águas

INEA – Instituto Estadual do Ambiente

IOW – Leibniz Institute for Baltic Sea Research Warnemünde

LMW – Low Molecular Weight

MGS – Median Grain Size

MO – Matéria Orgânica

MPS – Material Particulado em Suspensão

MRM – Multiple Reaction Monitoring

MS/MS – Tandem Mass Spectrometry

NIST – National Institute of Standards and Technology

NOAA – National Oceanic and Atmospheric Administration

OC – Organic Carbon

OM – Organic Matter

PAH – Polycyclic Aromatic Hydrocarbons

PCA – Principal Component Analysis

PCB – Polychlorinated Biphenyls e Bifenilos Policlorados

PEL – Probable Effect Level

POC – Pesticidas Organoclorados

POP – Persistent Organic Pollutants e Poluente Orgânico Persistente

PSR – Paraíba do Sul River

PTEC – Potentially Toxic Elements and Compounds

QL – Quantification Limit

RC – Rio Caeté

RESEX – Reserva Extrativista Marinha Caeté-Taperaçu

RPS – Rio Paraíba do Sul

SPT – Substâncias Potencialmente Tóxicas

TDS – Total Dissolved Solids

TEL – Threshold Effect Level

TN – Total Nitrogen

TOC – Total Organic Carbon

TSS – Total Suspended Solids

UENF – Universidade Estadual do Norte Fluminense Darcy Ribeiro

UFES – Universidade Federal do Espírito Santo

UFPA – Universidade Federal do Pará

UNEP – United Nations Environment Programme

USP – Universidade de São Paulo

$\delta^{13}\text{C}$ – Isótopo Estável de Carbono

$\delta^{15}\text{N}$ – Isótopo Estável de Nitrogênio

LISTA DE TABELAS

Capítulo 1:

Table 1. Land use and land cover in the past 40 years in the hydrographic basins of the Caeté River (Amazon Rainforest) and the Paraíba do Sul River (Atlantic Rainforest).....**Erro! Indicador não definido.**

Capítulo 2:

Capítulo 3:

Table 1. Geographical coordinates and characteristics of the watercourses in the PSR basin. Figure code represents the identification code in the figures, latitude and longitude are given in decimal degrees, and distance is measured from the confluence of the Paraitinga and Paraibuna rivers towards the ocean.90

Table 2. Global overview of decade-long temporal changes in water quality in the PSR Basin. 5th, 50th and 95th percentiles of 29 water quality parameters taken in the baseline period (2012–2014) and 2023 are contrasted (IF=median 2023 value/ median baseline value) and compared with national environmental standards (CONAMA 357/2005 Class 2 Water Bodies). Red values indicate parameters for which quantification limits (QL) varied over time; therefore, for these parameters reported IFs do not reflect true concentration increases.....94

LISTA DE FIGURAS

Figura 1: Representação conceitual das principais fontes antrópicas, vias de transporte e processos de transformação que controlam o aporte de matéria orgânica, nutrientes e contaminantes aos estuários tropicais.....2

Capítulo 1:

Fig. 1 Location of sediment and soil sampling sites at Caeté River (Northern Brazil, Amazon Rainforest) and Paraíba do Sul River (Southeastern Brazil, Atlantic Rainforest) estuaries.13

Fig. 2 Boxplots of OC and TN concentration (%), $\delta^{13}\text{C}_{\text{org}}$ (‰), $\delta^{15}\text{N}_{\text{total}}$ (‰), and (C:N)_a ratios in surface sediments and soils from estuarine and mangrove environments in the CR and PSR (Amazon and Atlantic Rainforests, respectively). Comparisons are shown by biome (1st column) and season (2nd and 3rd column). Significant differences are indicated above or below the boxplots with brackets: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant. When the interaction between factors is not significant, brackets represent only main effects. When the interaction is significant, multiple comparisons are performed among all factor combinations. Gray boxplots correspond to estuarine sediments only, as they reflect basin-scale changes.20

Fig. 3 Biplots of $\delta^{13}\text{C}_{\text{org}}$ (‰) vs. $\delta^{15}\text{N}_{\text{total}}$ (‰) (a) and posterior probability distributions of the proportional contribution of potential organic matter sources to sedimentary organic matter estimated using the Bayesian mixing model implemented in MixSIAR (b). Colored areas represent the mean and standard deviation of each end-member described in material and methods (e.g., C₃ and C₄ vegetation, sewage, marine sources). Violin plots represent the posterior density distributions of source contributions. The data were organized only with the results of $\delta^{13}\text{C}_{\text{org}}$ (‰) vs. $\delta^{15}\text{N}_{\text{total}}$ (‰).21

Fig. 4 Boxplots of PAHs, HCHs, DDTs and PCBs concentrations in surface sediments and soils from estuarine and mangrove environments in the CR and PSR (Amazon and Atlantic rainforest biomes, respectively). Comparisons are shown by biome (1st column) and season (2nd and 3rd column). Concentrations are given in ng g⁻¹. Significant differences are indicated above or below the boxplots with brackets: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant. When the interaction between factors is not significant, brackets represent only main effects. When the interaction is significant, multiple comparisons are performed among all factor

combinations. A logarithmic scale was applied to the y-axis for PAHs and PCBs concentration for better visualization. Gray boxplots correspond to estuarine sediments only.....24

Fig. 5 PAH diagnostic ratios used to infer the sources of PAHs in the estuarine sediment samples from the Caeté River and Paraíba do Sul River colored by DDT. Many samples are not shown in the figure due to PAH concentrations below QL, preventing ratio calculations.....25

Fig. 10 Variability in conductivity (a), dissolved Fe (b), phosphorus (c), and nitrate (d) along the Paraíba do Sul River and its tributaries in 2023. Sampling stations (1–56) are listed in Supplementary Materials 1 and 2; gray shading marks dams along the mainstem. For each parameter, the lower panel shows stations along the main course, while the upper panel shows tributary stations on the left (orange) and right (green) margins. Baseline conditions appear as a continuous red line in the lower panel, reflecting uninterrupted flow along the mainstem, and as separate red squares in the upper panel, corresponding to hydrologically independent tributaries.**Erro! Indicador não definido.**

Capítulo 2:

Fig.: 1 Design of sediments samples across the Caeté River (CR) and Paraíba do Sul River (PSR) basin.....54

Fig.: 2 Concentrations of potential pollutants in surface sediment and soil. Left column: comparison between basins (CR and PSR); center and right columns: seasonal variation (dry and rainy) by environment (estuary and mangrove) in the CR and PSR basins, respectively. When the interaction between environment and season was not significant, factors were analyzed separately: lowercase letters indicate differences between environments and uppercase letters indicate differences between seasons. When the interaction was significant, lowercase letters denote pairwise differences among all factor combinations. n.s: no significance was observed. When necessary, Y-axis was log-transformed for better visualization.....61

Fig.: 3 Offspring production of *Nitokra* sp. across sampling environments in the Caeté River (a) and Paraíba do Sul River (b) basins. Boxplots represent observed data. Letters denote sig-nificant differences among all Basin × Environment combinations based on pa.....62

Fig.: 4 Mixture effect estimates (Ψ) for each contaminant class and the cross-class mixture model estimated by quantile g-computation. Points represent Ψ values with 95% confidence intervals; percentages indicate the estimated change in relative reproductive output per simultaneous one-quartile increase in all contaminants within the class, back-transformed as $[\exp(\Psi) - 1] \times 100$. Color indicates statistical significance: red, $p < 0.05$; orange, $p < 0.10$; gray, not significant.	64
Fig.: 5 Convergence analysis integrating quantile g-computation, Elastic Net regression, and PCA for the identification of individual toxicity drivers within each contaminant class. The left panel displays the individual proportional weights (w_j) from adjusted.....	65

Capítulo 3:

Figure 1 Water quality sampling stations in the Paraíba do Sul River Basin, Southeastern Brazil. The figure was prepared using the software ArcGIS (ArcMap 10.8 version 10.7.0.10450; https://desktop.arcgis.com/en/).	89
Figure 2 Magnitude of CONAMA 357 violations for environmental parameters along the main course of the Paraíba do Sul River (a) and its subdivisions (b–d) in 2023. Boxplots show the ratio between measured values and the national water-quality standard; ratios >1 indicate non-compliance. Note that (i) DO violations correspond to values <i>below</i> the standard ($DO < 1$), and (ii) pH violations occur when values fall <i>below</i> the lower limit ($pH_{lower} > 1$) or <i>above</i> the upper limit ($pH_{upper} < 1$). QLS for total As, Hg, and Cd exceed the standards, preventing a meaningful compliance assessment. <i>E. coli</i> in the upper section was measured only at one HUWRM near the middle section (see Supplementary Material 2). Variables are ordered by increasing median violation magnitude in all panels.....	97
Figure 3 Magnitude of baseline violations for different environmental parameters along the principal course of the Paraíba do Sul River and its subdivisions. Boxplots represent the distributions of the ratios of recorded values; values above 1 indicate that the parameters exceeded the baseline established during the reference period (2012–2014), signaling a decrease in water quality over the last decade	99
Figure 4 Temporal trends (left) and seasonal cycles (right) of dissolved Fe (a) and total phosphorus (b) from 2012 to 2023 in the main course of the Paraíba do Sul River. The	

horizontal blue line represents the threshold established by CONAMA 357 legislation. The black line on the Paraíba do Sul section is the fitted regression model. 101

Figure 5 Variability in conductivity (a), dissolved Fe (b), phosphorus (c), and nitrate (d) along the Paraíba do Sul River and its tributaries in 2023. Sampling stations (1–56) are listed in Supplementary Materials 1 and 2; gray shading marks dams along the mainstem. For each parameter, the lower panel shows stations along the main course, while the upper panel shows tributary stations on the left (orange) and right (green) margins. Baseline conditions appear as a continuous red line in the lower panel, reflecting uninterrupted flow along the mainstem, and as separate red squares in the upper panel, corresponding to hydrologically independent tributaries. 103

Figure 6 Principal Component Analysis (PCA) of water quality variables in the PSR basin, including metal concentrations, physicochemical parameters, and biological indicators. Panels show the baseline period (a) and 2023 data (b). Ellipses represent 68% confidence intervals for each basin sector. 105

RESUMO

Os estuários integram os processos físicos, químicos e biológicos que ocorrem em toda a bacia hidrográfica, acumulando registros das atividades humanas desenvolvidas a montante e constituindo importantes indicadores da qualidade ambiental de ecossistemas costeiros. No Brasil, onde se concentram a maior extensão de florestas tropicais e a segunda maior área de manguezais do mundo, a expansão agrícola, a urbanização, a industrialização e a histórica deficiência em saneamento básico têm alterado a dinâmica da matéria orgânica (MO), favorecido o aporte de contaminantes e comprometido a qualidade desses ambientes. Nesse contexto, esta tese teve como objetivo avaliar os efeitos das pressões antrópicas sobre a biogeoquímica da MO, a contaminação e a toxicidade dos sedimentos estuarinos, bem como sobre a qualidade da água em bacias hidrográficas tropicais. O primeiro capítulo investigou como diferentes históricos de uso e ocupação do solo influenciam a composição isotópica da MO sedimentar e a distribuição de poluentes orgânicos persistentes nos estuários dos rios Caeté (RC, Amazônia) e Paraíba do Sul (RPS, Mata Atlântica). Os resultados evidenciaram assinaturas biogeoquímicas contrastantes entre as bacias: no RC, apesar da recente expansão agropecuária, a MO sedimentar permaneceu predominantemente derivada de vegetação C3, enquanto o perfil de contaminação refletiu o uso histórico de pesticidas organoclorados; no RPS, o esgoto doméstico foi identificado como a principal fonte de MO, e elevadas concentrações de PAHs e PCBs de alto peso molecular evidenciaram décadas de intensa pressão urbana e industrial. A partir desse diagnóstico, o segundo capítulo avaliou o potencial ecotoxicológico dos sedimentos por meio de bioensaios crônicos com o copépodo bentônico *Nitokra* sp. associados à modelagem estatística de misturas de contaminantes. Os resultados demonstraram que o aumento simultâneo das concentrações de PCBs, pesticidas organoclorados (OCPs) e PAHs de alto peso molecular constitui o principal determinante da redução da produção reprodutiva, evidenciando a importância de abordagens baseadas em misturas de contaminantes em relação à avaliação de compostos isolados. O terceiro capítulo ampliou a investigação para a escala da bacia hidrográfica, analisando dez anos de monitoramento da qualidade da água no RPS. Foi observada deterioração progressiva de múltiplos parâmetros físicos, químicos e biológicos, com destaque para o aumento das concentrações de ferro dissolvido e fósforo total e para recorrentes violações dos padrões estabelecidos pela legislação brasileira. Em conjunto, os resultados demonstram que as mudanças no uso e ocupação do solo modificam a biogeoquímica da matéria orgânica, favorecem o acúmulo de contaminantes, comprometem a qualidade da água e representam risco ecotoxicológico para a biota bentônica, com efeitos reprodutivos detectáveis mesmo em concentrações ambientalmente relevantes. Esses achados reforçam a importância de abordagens integradas, multidisciplinares e de longo prazo para compreender os impactos das atividades humanas e subsidiar a gestão de bacias hidrográficas e ecossistemas costeiros tropicais.

Palavras-chave: contaminação ambiental, ecotoxicologia, metais, POPs.

ABSTRACT

Estuaries integrate the physical, chemical, and biological processes occurring throughout river basins, accumulating records of upstream human activities and serving as important indicators of the environmental quality of coastal ecosystems. In Brazil, which hosts the world's largest extent of tropical forests and the second-largest mangrove area, agricultural expansion, urbanization, industrialization, and the historical lack of adequate sanitation have altered organic matter (OM) dynamics, increased contaminant inputs, and compromised the environmental quality of these systems. In this context, this thesis aimed to evaluate the effects of anthropogenic pressures on OM biogeochemistry, sediment contamination and toxicity, as well as water quality in tropical river basins. The first chapter investigated how contrasting land-use histories influence the isotopic composition of sedimentary OM and the distribution of persistent organic pollutants in the estuaries of the Caeté River (CR, Amazon Rainforest) and the Paraíba do Sul River (PSR, Atlantic Rainforest). The results revealed contrasting biogeochemical signatures between the two basins. In the CR, despite recent agricultural expansion, sedimentary OM remained predominantly derived from C3 vegetation, whereas the contamination profile reflected the historical use of organochlorine pesticides. In the PSR, domestic sewage was identified as the primary source of OM, while elevated concentrations of high-molecular-weight PAHs and PCBs reflect decades of intense urban and industrial pressure. Based on this environmental characterization, the second chapter evaluated the ecotoxicological potential of estuarine sediments through chronic toxicity tests with the benthic copepod *Nitokra* sp. combined with contaminant mixture modeling. The results demonstrate that the simultaneous increase in PCB, organochlorine pesticide (OCP), and high-molecular-weight PAH concentrations constitutes the main determinant of reduced reproductive output, highlighting the importance of mixture-based approaches over assessments based on individual contaminants. The third chapter expanded the investigation to the river-basin scale by analyzing a ten-year water quality monitoring dataset from the Paraíba do Sul River Basin. Progressive deterioration was observed across multiple physical, chemical, and biological water quality parameters, particularly increasing dissolved iron and total phosphorus concentrations, as well as recurrent violations of Brazilian environmental standards. Overall, the results demonstrate that land-use change alters OM biogeochemistry, promotes contaminant accumulation, degrades water quality, and poses ecotoxicological risks to benthic biota, with measurable reproductive effects occurring even at environmentally relevant concentrations. These findings reinforce the importance of integrated, multidisciplinary, and long-term approaches to understand the impacts of human activities and to support the assessment and management of tropical river basins and coastal ecosystems.

Keywords: ecotoxicology, environmental contamination, metals, POPs.

ESTRUTURA DA TESE

Esta tese foi estruturada de forma sequencial, de modo que cada capítulo responde a uma etapa específica da investigação e fornece a base para o capítulo seguinte. Em conjunto, os três capítulos integram diferentes abordagens para compreender como as mudanças no uso e ocupação do solo influenciam a biogeoquímica da matéria orgânica, o acúmulo de contaminantes, os efeitos ecotoxicológicos e a qualidade da água em ecossistemas aquáticos tropicais.

1. Uma introdução geral, que apresenta a fundamentação teórica que sustenta a pesquisa, abordando as principais pressões antrópicas sobre bacias hidrográficas tropicais, os processos de transporte e deposição de matéria orgânica e contaminantes nos estuários, as ferramentas biogeoquímicas e ecotoxicológicas utilizadas para avaliação desses sistemas e as lacunas de conhecimento que motivaram o desenvolvimento desta tese.
2. Três capítulos redigidos em formato de artigos científicos, que abordam diferentes dimensões da influência do uso e ocupação do solo sobre a dinâmica biogeoquímica, a contaminação ambiental e os impactos ecotoxicológicos em sistemas aquáticos tropicais.
 - O Capítulo 1 estabelece o contexto biogeoquímico das áreas de estudo, avaliando como diferentes históricos de uso e ocupação do solo influenciam a composição isotópica da matéria orgânica sedimentar e a distribuição de poluentes orgânicos persistentes (PAHs, HCHs, DDTs, PCBs) nos estuários dos Rios Caeté (Pará) e Paraíba do Sul (Rio de Janeiro). Os resultados permitem identificar as principais assinaturas ambientais associadas às diferentes pressões antrópicas existentes nas duas bacias hidrográficas.
 - O Capítulo 2, com base na caracterização ambiental apresentada no capítulo 1, investiga os determinantes da toxicidade sedimentar em estuários tropicais utilizando o copépodo bentônico *Nitokra* sp. como organismo-teste, a partir de bioensaios crônicos avaliando a produção reprodutiva. A caracterização química dos sedimentos (metais traço, PAHs, PCBs e agrotóxicos organoclorados) foi combinada a uma abordagem hierárquica de modelagem de misturas de contaminantes para identificar quais compostos, dentro de misturas complexas, são os principais responsáveis pelos efeitos tóxicos observados.
 - O Capítulo 3 amplia a escala de análise para toda a bacia hidrográfica do Rio Paraíba do Sul utilizando de uma série histórica para investigar a dinâmica espaço-temporal de parâmetros físicos, químicos e biológicos da qualidade da água na bacia do Rio Paraíba do Sul ao longo de uma série histórica,

identificando tendências temporais, padrões sazonais, gradientes espaciais de contaminação e os principais fatores associados à degradação ambiental.

Cada capítulo apresenta resumo, introdução, material e métodos, resultados, discussão, conclusão e referências, estruturados de acordo com as normas dos periódicos científicos nos quais os manuscritos foram submetidos.

3. Integram os resultados obtidos nos três capítulos, discutindo suas contribuições para a compreensão dos efeitos das mudanças no uso do solo sobre ecossistemas aquáticos tropicais, bem como suas implicações para o monitoramento ambiental e a gestão de bacias hidrográficas.
4. As referências bibliográficas, apresentam as referências utilizadas na Introdução Geral e nas Considerações Finais, enquanto cada capítulo possui sua própria lista de referências, conforme as normas dos periódicos para os quais os manuscritos foram preparados

INTRODUÇÃO GERAL

O crescimento populacional, a expansão das atividades industriais e agrícolas e a urbanização acelerada têm modificado as características biogeoquímicas dos ambientes terrestres e aquáticos. Esse processo implica a substituição de florestas nativas por áreas antrópicas e, conseqüentemente, a introdução de substâncias potencialmente tóxicas (SPT) nos ecossistemas (Tanaka et al., 2021; Gerson, 2025). Tal conversão é particularmente crítica em florestas tropicais, que apresentam as maiores taxas de perda. O Brasil apresenta a maior extensão de floresta primária úmida tropical do mundo e, entre 2001 e 2014, perdeu cerca de 5,5% de sua cobertura original (Turubanova et al., 2018; Tanaka et al., 2021). A perda desses ecossistemas altera a dinâmica da matéria orgânica (MO) e intensifica processos erosivos, favorecendo o transporte de nutrientes e contaminantes para os sistemas aquáticos por escoamento superficial (Tanaka et al., 2021).

Entretanto, os efeitos das mudanças no uso e ocupação do solo extrapolam o ambiente terrestre e propagam-se por toda a bacia hidrográfica. Diferentes atividades antrópicas promovem o aporte de MO, nutrientes e diversas classes de contaminantes, que são transportados ao longo da bacia por processos hidrológicos e sedimentares até alcançarem os ecossistemas costeiros (Figura 1). Dessa forma, os estuários atuam como integradores dos processos físicos, químicos e biológicos que ocorrem na bacia hidrográfica, registrando em alterações simultâneas na qualidade da água, sedimento e ambiental (Tanaka et al., 2021).

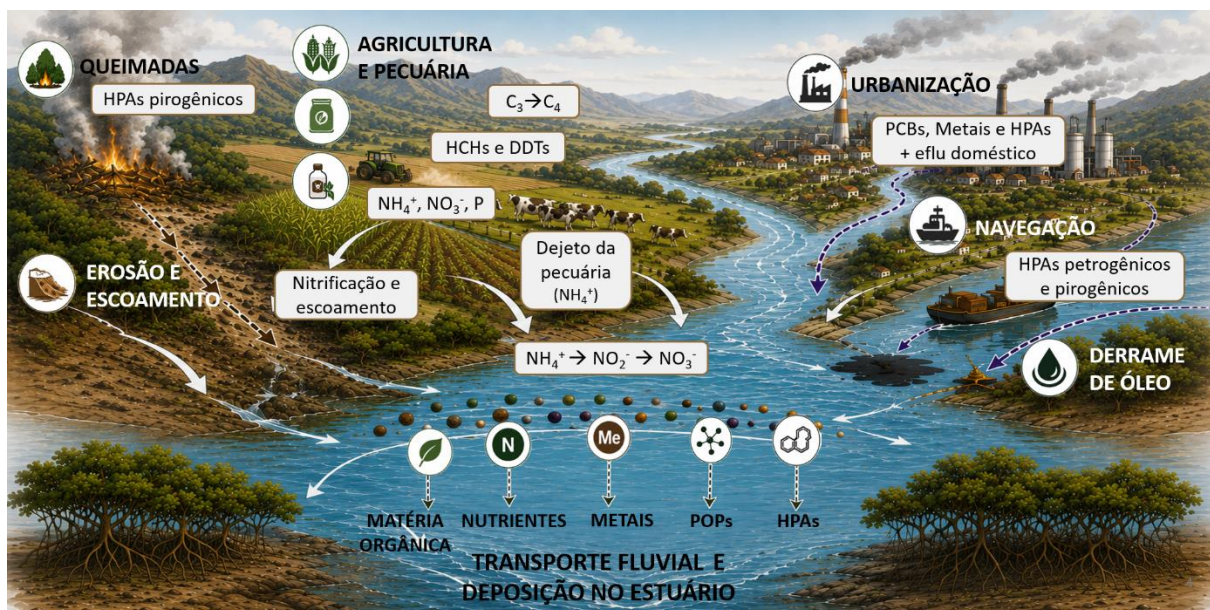


Figura 1: Representação conceitual das principais fontes antrópicas, vias de transporte e processos de transformação que controlam o aporte de matéria orgânica, nutrientes e contaminantes aos estuários tropicais.

A natureza e a origem dessas substâncias variam conforme o tipo de uso do solo e ocupação humana predominante na bacia. As práticas agrícolas e a mineração são responsáveis pelo aporte difuso de pesticidas organoclorados (POCs) como os Dicloro-Difenil-Tricloroetano (DDTs) e Hexaclorocicloexano (HCHs), além de metais traço oriundos do processamento mineral e rejeitos de mineração (Barhoumi et al., 2019; Quaresma et al., 2021; Vergilio et al., 2021). Paralelamente, o lançamento direto de efluentes domésticos e industriais nos corpos hídricos, representam uma rota pontual de contaminação, introduzindo elevadas cargas de MO, micro-organismos patogênicos, metais e outros poluentes orgânicos persistentes (POPs), como os hidrocarbonetos policíclicos aromáticos (HPAs) e os bifenilos policlorados (PCBs; Pfeiffer et al., 1986; Barhoumi et al., 2019; Pereira et al., 2021; Swain, 2024). O transporte atmosférico, por sua vez, representa uma rota crítica de dispersão de SPT, permitindo que POPs e metais voláteis, como Hg, ou aqueles associados a partículas, como Pb, alcancem sistemas aquáticos geograficamente remotos das fontes emissoras (Gerson, 2025; Swain, 2024; Serafim et al., 2026).

Uma vez nos sistemas aquáticos, o principal vetor de transporte de partículas a jusante é o material particulado em suspensão (MPS). Devido às suas características hidrofóbicas, contaminantes orgânicos como HPAs, PCBs e POCs tendem a se associar fortemente à MO particulada e ao MPS. No caso dos metais, a

capacidade de adsorção depende diretamente das propriedades físicas e químicas das partículas. As frações finas, como siltes e argilas, apresentam elevada área superficial específica, alta capacidade de troca catiônica (CTC) e predominância de cargas superficiais negativas, características que favorecem a retenção de cátions metálicos MO. Além disso, óxidos e hidróxidos de ferro e manganês, bem como a MO associada ao MPS, desempenham papel fundamental na adsorção e complexação de metais traço, aumentando sua capacidade de sorção e transporte nos sistemas aquáticos (Horowitz, 1991; Jones, 1999; Bayen, 2012). Durante esse transporte, partículas mais grosseiras tendem a se depositar nos trechos fluviais, enquanto partículas finas permanecem em suspensão e são transportadas para regiões estuarinas, acumulando-se progressivamente nos sedimentos através de processos de floculação e retenção mecânica associada à estrutura de raízes dos manguezais (Molisani et al., 1999). Nesse contexto, estuários e manguezais atuam como importantes zonas de retenção e transformação de MO e contaminantes provenientes da bacia hidrográfica a montante (Lacerda et al., 1993; Doong et al., 2008; Serafim et al., 2023). Consequentemente, os sedimentos estuarinos constituem arquivos ambientais capazes de integrar processos que ocorrem em diferentes escalas espaciais e temporais. Diferentemente da coluna d'água, que responde rapidamente a eventos hidrológicos e descargas pontuais (Ovalle et al., 2013), os sedimentos preservam registros históricos das alterações promovidas pelo uso e ocupação do solo, permitindo reconstruir a evolução da contaminação e das mudanças biogeoquímicas ao longo do tempo (Serafim et al., 2023).

Os manguezais são ecossistemas de zonas entre-marés tropicais e subtropicais que ocupam aproximadamente 83 a 138 mil km² de faixas costeiras descontínuas (Call et al., 2019). Esses ambientes desempenham importantes funções ecológicas, incluindo elevada produtividade primária, ciclagem de nutrientes, regulação climática e suporte à reprodução e ao desenvolvimento de espécies aquáticas (ICMBio, 2018).

O Brasil, além de abrigar a maior extensão de floresta tropical do mundo, também possui a segunda maior área de manguezais, ecossistemas fortemente ameaçados pela expansão humana, especialmente para atividades de aquicultura e agricultura (ICMBio, 2018; Turubanova et al., 2018; Bunting et al., 2022; UNDRR, 2023). Os impactos gerados na bacia, uma vez registrados nos sedimentos estuarinos, podem ser rastreados por ferramentas geoquímicas e ecotoxicológicas

(Serafim et al., 2023; Silva et al., 2023). Isótopos estáveis de carbono ($\delta^{13}\text{C}$) e nitrogênio ($\delta^{15}\text{N}$) são comumente utilizados como traçadores das fontes de MO (Thornton e McManus, 1994; Martinelli et al., 2020), enquanto metais traço e POPs atuam como marcadores geoquímicos das atividades agrícolas, urbanas e industriais (Oliveira et al., 2016; Sreedevi et al., 2023; Swain, 2024). Dessa forma, embora os marcadores geoquímicos permitam identificar fontes de MO e rastrear contaminantes, eles não respondem se essas alterações efetivamente representam risco para os organismos aquáticos. A avaliação integrada da qualidade ambiental exige, portanto, ferramentas capazes de conectar a caracterização química dos sedimentos às respostas biológicas dos organismos expostos.

Os ensaios ecotoxicológicos, por sua vez, vêm sendo empregados ao longo dos anos com intuito de demonstrar o potencial de matrizes ambientais de induzir efeitos tóxicos em organismos de diferentes níveis tróficos, uma vez que as concentrações por si só não fornecem informações quanto à bioacumulação e potencial toxicidade para os organismos (Silva et al., 2023). Nesse contexto, copépodos do gênero *Nitokra* destacam-se como organismos-teste particularmente adequados para a avaliação de sedimentos tropicais, por habitarem a interface sedimento-água, apresentarem ciclo de vida curto, fácil cultivo em laboratório e elevada sensibilidade a contaminantes, além de serem representativos da fauna bentônica de regiões costeiras tropicais (Lotufo e Abessa, 2002; Ward et al., 2011). Apesar da consolidação dos ensaios ecotoxicológicos como ferramenta diagnóstica, a identificação dos compostos responsáveis pela toxicidade permanece um dos maiores desafios da ecotoxicologia ambiental, especialmente quando essas SPT ocorrem simultaneamente em misturas complexas. Abordagens estatísticas recentes, como o quantile g-computation e a regressão Elastic Net, oferecem alternativas robustas para estimar efeitos combinados de misturas de contaminantes mesmo em condições de alta colinearidade (Keil et al., 2020; Yu et al., 2022), representando um avanço metodológico ainda pouco explorado na ecotoxicologia de sedimentos tropicais.

Além da avaliação integrada entre geoquímica e ecotoxicologia, compreender os efeitos das atividades humanas exige expandir a análise para além dos sedimentos estuarinos. As mesmas pressões que promovem o acúmulo de contaminantes nesses ambientes também alteram continuamente a qualidade da água ao longo da rede

hidrográfica, modificando processos físicos, químicos e biológicos em diferentes escalas espaciais e temporais. A avaliação integrada da qualidade ambiental de bacias hidrográficas requer, além do diagnóstico em sedimentos e biota, o monitoramento contínuo de parâmetros físicos, químicos e biológicos da água ao longo do tempo. Variações pontuais são insuficientes para capturar tendências de deterioração, padrões sazonais e gradientes espaciais de contaminação que somente séries históricas de longo prazo conseguem revelar (Ovalle et al., 2013; Souza et al., 2026).

Apesar dos avanços no uso de marcadores isotópicos, contaminantes orgânicos persistentes, metais e bioensaios ecotoxicológicos, poucos estudos integraram essas diferentes abordagens em uma estrutura capaz de relacionar, simultaneamente, as mudanças no uso do solo, o transporte de MO e contaminantes, seus efeitos sobre a biota e as alterações na qualidade da água em escala de bacia hidrográfica. Essa lacuna limita a compreensão dos mecanismos que conectam as pressões antrópicas aos impactos observados nos ecossistemas aquáticos tropicais.

Diante desse contexto, parte-se da hipótese de que diferentes históricos de uso e ocupação do solo produzem assinaturas biogeoquímicas distintas nos sedimentos estuarinos, modificam a composição e o transporte da MO, favorecem o acúmulo diferencial de contaminantes e resultam em diferentes níveis de risco ecotoxicológico para a biota, refletindo-se também na qualidade da água ao longo da bacia hidrográfica.

Para testar essa hipótese, esta tese foi estruturada em três capítulos complementares e interdependentes. O primeiro estabelece o contexto biogeoquímico dos sistemas estudados ao investigar como diferentes históricos de uso e ocupação do solo influenciam a composição isotópica da MO sedimentar e o acúmulo de POPs nos estuários dos Rios Caeté e Paraíba do Sul. A partir desse diagnóstico, o segundo capítulo avalia se esses contaminantes representam risco para a biota bentônica, empregando bioensaios crônicos com *Nitokra* sp. combinados à modelagem estatística de misturas de contaminantes para identificar os principais determinantes da toxicidade reprodutiva. Por fim, o terceiro capítulo amplia a escala de investigação para toda a bacia do rio Paraíba do Sul, analisando uma série histórica de dez anos de monitoramento para identificar tendências temporais, gradientes espaciais e os principais fatores associados à degradação da qualidade da água. Em conjunto, esses

capítulos estabelecem uma sequência lógica que conecta as mudanças no uso do solo aos processos biogeoquímicos, aos efeitos ecotoxicológicos e à qualidade ambiental de ecossistemas aquáticos tropicais.

CAPÍTULO 1:

Land-use impacts on Amazon and Atlantic Rainforest coastal ecosystems revealed by multiple biogeochemical markers

Submetido na **Marine Pollution Bulletin**

Qualis-Capes (Área de Biodiversidade, 2021-2024): A1

Fator de Impacto (2025): 4.9

Status: **PUBLICADO** <https://doi.org/10.1016/j.marpolbul.2026.119899>

CAPÍTULO 1 - Land-use impacts on Amazon and Atlantic Rainforest coastal ecosystems revealed by multiple biogeochemical markers

Échily Sartori^{a*}, Cristiane dos Santos Vergilio^b, Tassiana S. G. Serafim^c, Diego Lacerda^a, Luiza Silva do Nascimento^a, Pedro Vianna Gatts^a, Mariana Freitas^a, Jamile Tamer Nasser^d, Marcelo Gomes de Almeida^a, César C. Martins^d, Nils E. Asp^e, Carlos Eduardo de Rezende^{a,*}

^a Grupo de Pesquisa em Biogeoquímica de Ecossistemas Aquáticos, Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Avenida Alberto Lamego, 2000, Parque Califórnia, Campos dos Goytacazes, Rio de Janeiro, 28013-602, Brazil.

^b Laboratório de Ecotoxicologia, Departamento de Biologia, Centro de Ciências Exatas Naturais e da Saúde, Universidade Federal do Espírito Santo – Campus Alegre (UFES). Alto Universitário, S/N, Alegre, Espírito Santo, 29500-000, Brazil.

^c Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Seestraße 15, 18119, Rostock, Germany.

^d Laboratório de Química Orgânica Marinha, Instituto Oceanográfico, Universidade de São Paulo (USP), Praça do Oceanográfico, 191, Cidade Universitária, São Paulo, 05508-120, Brazil.

^e Instituto de Estudos Costeiros, Universidade Federal do Pará (UFPA), Alam. Leandro Ribeiro, S/N, Bairro Aldeia, Campus UFPA, Bragança, Pará, 68600-000, Brazil.

Abstract

Land-use changes were assessed using isotopic and elemental composition of organic matter (OM) and organic pollutants in estuarine sediments from two Brazilian basins: the Caeté River (CR, Amazon Rainforest) and the Paraíba do Sul River (PSR, Atlantic Rainforest). Between 1985 and 2024, anthropogenic land use in CR increased in 39%, while PSR decreased by 2.6%. CR sediments exhibited lower $\delta^{13}\text{C}_{\text{org}}$ ($-27.3 \pm 0.9\text{‰}$) and $\delta^{15}\text{N}_{\text{total}}$ ($2.1 \pm 2.2\text{‰}$) than PSR ($-23.6 \pm 2.9\text{‰}$ and $5.6 \pm 1.0\text{‰}$, respectively). Seasonal variability occurred only in CR, with more ^{13}C -depleted and ^{15}N -enriched values in the rainy season, suggesting surface runoff of C_3 -derived OM from rainforest or mangroves and upstream anthropogenic inputs. The Bayesian mixing model (MixSIAR) indicated clear contrasts in OM sources between basins. In the CR basin, sedimentary OM was predominantly derived from mangroves (median = 0.76), with a secondary contribution from sewage (0.18). In contrast, in the PSR basin, sewage was the dominant OM source (0.65), followed by mangrove contributions (0.28). Organic

pollutants correlated with OC, showing that contaminant distribution is partly governed by OM dynamics. PAH diagnostic ratios revealed the sewage, biomass/coal burning as the main source in PSR, with additional fossil-fuel inputs, while CR reflected mixed combustion sources linked to agricultural fires and boat traffic. Organochlorine patterns in CR suggest historical pesticide use and diffuse atmospheric input, while PSR shows lindane and local/industrial influences. These results highlight contrasting OM dynamics under different land-use intensities and the dual role of estuaries in storing carbon and pollutants.

Keywords: Caeté River, Mangrove, Paraíba do Sul River, PAH, POP, Stable Isotopes.

Introduction

Brazil hosts the world's largest area of native tropical forests (Turubanova et al., 2018), and the second largest mangrove (Bunting et al., 2022). These ecosystems regulate climate, sequester carbon, maintain hydrological balance, and shelter biodiversity (Pinto et al., 2023). However, land-use expansion, mainly livestock, has intensified deforestation, replacing native vegetation with agriculture, pasture, and secondary forests, placing Brazil among the nations with greatest tropical forest loss (Lapola et al., 2014; Pinto et al., 2023).

The Amazon and Atlantic Rainforests hold global importance but differ in conservation status (Pinto et al., 2023). The Atlantic retains only 12% of its original cover, now fragmented (Lapola et al., 2014), while the Amazon still harbors the largest continuous tropical forest, crucial for global climate regulation, though deforestation has surged from livestock, logging, mining, and urbanization (Lapola et al., 2014). Both biomes are highly biodiverse, but the Atlantic shows greater endemism (Broggio et al., 2024) and illustrates potential future scenarios for the Amazon under similar pressures.

Replacing native forests with pastures alters soil C and N stocks, increases erosion and runoff, and mobilizes organic matter (OM) toward river systems (Serafim et al., 2023; Dow et al., 2024). During transport, OM is transformed through leaching, mineral associations, photochemical alteration, and microbial degradation, processes that preferentially mineralize labile, nutrient-rich compounds and enrich the remaining pool in refractory material and heavier isotopes (Ward et al., 2017; Bianchi et al., 2018). The preservation of these isotopic signatures is further modulated by sediment granulometry, where fine-grained fractions promote mineral-organic associations that

protect OM from rapid mineralization (Keil and Mayer, 2014). Stable isotopes ($\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{total}}$) and the $(\text{C:N})_{\text{a}}$ are widely used to trace these sources and transformation pathways. Native tropical forests dominated by C_3 -plants, show lower $\delta^{13}\text{C}_{\text{org}}$ values (-32.0 to -28.6‰) and moderate $\delta^{15}\text{N}_{\text{total}}$ (4.4 to 7.7‰) (Ometto et al., 2006; Ribas, 2012; Martinelli et al., 2020), whereas land-use change alters OM composition (Bernardes et al., 2004). While $\delta^{13}\text{C}_{\text{org}}$ mainly reflects vegetation type, $\delta^{15}\text{N}_{\text{total}}$ is more sensitive to land-use practices (Lizaga et al., 2021; Vezzone et al., 2021; Park et al., 2023). Pastures and croplands dominated by C_4 -plants display higher $\delta^{13}\text{C}$ values (-15 to -10‰) (Bouillon et al., 2011; Ribas, 2012), while manure and sewage inputs enrich $\delta^{15}\text{N}$ through nitrification and denitrification (Li et al., 2023). In aquatic systems, terrestrial OM mixes with autochthonous and mangrove- C_3 inputs, forming isotopic gradients along the land–ocean continuum (Thornton and McManus, 1994; Serafim et al., 2023).

Estuaries, transitional zones between land and sea, retain, transform, and export OM via remineralization, photodegradation, flocculation, and sorption, preserving refractory fractions (Eisma et al., 1994; Lämmle et al., 2022; Serafim et al., 2023). Their sediments reflect combined terrestrial, anthropogenic, and marine inputs. Within estuarine systems, mangroves act as biogeochemical hotspots with large carbon stocks, intense OM cycling, and export of OM (Bouillon et al., 2008; Santos-Andrade et al., 2021; Zhao et al., 2024).

Molecular markers complement isotopic tracers by identifying anthropogenic inputs. Polycyclic aromatic hydrocarbons (PAHs) indicate contributions from sewage, biomass and fossil fuel burning, and petroleum sources (Nam et al., 2008; Martins et al., 2010; Gurgatz et al., 2023). Persistent organic pollutants (POPs) such as the pesticides hexachlorocyclohexane (HCH) and dichlorodiphenyltrichloroethane (DDT), as well as the industrial chemical compounds polychlorinated biphenyls (PCBs), trace agricultural and urban-industrial runoff, respectively (Nam et al., 2008; Oliveira et al., 2016; Mao et al., 2021). Their hydrophobicity promotes strong adsorption to OM, coupling their transport and accumulation to the same processes controlling OM export from drainage basins to estuarine sediments (Cornelissen et al., 2005; Bianchi et al., 2018). Thus, changes in land use simultaneously affect (i) the sources and isotopic composition of OM and (ii) the type and magnitude of contaminant inputs, linking both proxies to common watershed-scale processes. Interpreted together with land-use trajectories, these complementary proxies provide a framework to investigate how

human activities influence both OM dynamics and contaminant accumulation in estuarine sediments.

Despite extensive research on tropical estuaries, most studies have addressed either OM sources using stable isotopes or contaminant distributions using molecular markers, rarely integrating both approaches. This limitation constrains the understanding of how long-term land-use trajectories influence the coupled dynamics of OM composition and pollutant accumulation in estuarine sediments. Here, we investigate land-use and cover changes (1985–2024) in two contrasting Brazilian basins within the Amazon and Atlantic Rainforests and evaluate their influence on $\delta^{13}\text{C}_{\text{org}}$, $\delta^{15}\text{N}_{\text{total}}$, (C:N)_a, PAHs and POPs in estuarine sediments and mangrove soils along the land–ocean continuum. We hypothesize that basins with contrasting land-use histories will exhibit coherent shifts between isotopic signatures and contaminant profiles, reflecting the joint control of vegetation change, anthropogenic emissions, and OM-associated transport processes.

Material and Methods

Amazon Rainforest hydrographic basin

Located in northeastern Pará State, the Caeté River (CR) basin represents the Amazonian system investigated in this study, covering ~2,230 km² with a 149 km main channel flowing from its headwaters in Bonito to the Caeté Urumajó Bay. Part of the basin lies within the Caeté Taperaçu Marine Extractive Reserve (RESEX) and includes seven municipalities and 19 traditional communities (Abdala et al., 2012; Gomes et al., 2013; Asp et al., 2018; Pereira et al., 2021).

The CR basin exhibits a markedly seasonal climate, with rainfall concentrated in the first half of the year and a pronounced dry period in the last quarter. According to the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), average annual precipitation is 2,755 mm, with a dry season in October–November (near zero rainfall), and a rainy season from December to August, peaking in March. The CR shows a mean discharge of ~50 m³·s⁻¹, ranging from 7 m³·s⁻¹ in November to 90 m³·s⁻¹, usually in April (Asp et al., 2018). Although other studies delimit the seasons slightly differently (Dittmar et al., 2001; Gomes et al., 2013), they all agree on high annual rainfall and a strongly seasonal hydrological regime. The system is macro-tidal, with spring tides about 5 m (Dittmar et al., 2001; Asp et al., 2018).

Although sparsely populated, the basin faces socio environmental challenges due to inadequate sanitation and resource exploitation encouraged since the 1970s. Issues include open solid waste disposal, lack of sewage treatment, and pressures from agro-industrial, logging, mining, and fishing activities (Gorayeb et al., 2009, 2011; Guimarães, 2011).

Atlantic Rainforest hydrographic basin

The Paraíba do Sul River (PSR) basin is in southeastern Brazil, the country's most densely populated and industrialized region. It covers approximately 56,927 km², with the main river extending 1,100 km from the confluence of the Paraíba and Paraitinga Rivers to its mouth in São João da Barra, Rio de Janeiro State (Dittmar et al., 2012; Serafim et al., 2023). The basin supplies water to several urban centers (ANA, 2011). The climate is strongly seasonal, with dry period from April to early October and rainy period from late October to March, with average river discharge ranging from 200 to 2600 m³·s⁻¹ (Marques et al., 2017; Cotovicz et al., 2020; Neves et al., 2021; Serafim et al., 2023). Although studies delimit the dry and rainy seasons differently, they all agree that the region exhibits a strong seasonal climate.

The PSR basin is heavily impacted by industrial effluents and untreated domestic sewage (Pfeiffer et al., 1986; AGEVAP, 2020). Intensive agriculture and livestock production also contribute to environmental pressures, historically including the use of organomercury-based fungicide, which have increased mercury and other metal loads. The basin has additionally experienced several environmental accidents involving industrial spills and contaminant releases (Álvarez & Valpassos, 2023). More recently, reports of illegal gold mining have introduced additional mercury inputs to the system (Gomes et al., 2022).

Field sampling

Surface sediment and soil samples from estuarine/coastal and mangrove areas were collected in the PSR (Atlantic Rainforest) and CR (Amazon Rainforest) basins between 2022 and 2023, during both dry and rainy seasons, totaling 54 samples (Fig. 1). Half were obtained along a salinity gradient (0–36 PSU), and the remainder from adjacent mangrove areas (Table S1). Samples were transported in thermal boxes and frozen at -20 °C upon arrival for analysis.

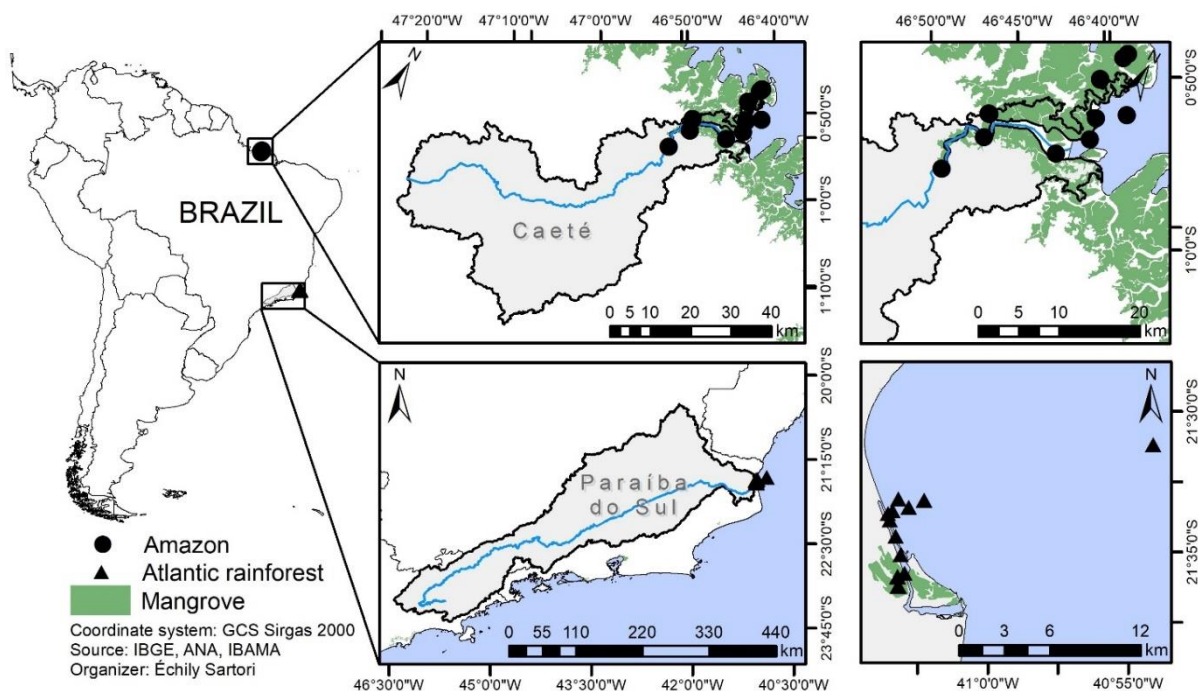


Fig. 1 Location of sediment and soil sampling sites at Caeté River (Northern Brazil, Amazon Rainforest) and Paraíba do Sul River (Southeastern Brazil, Atlantic Rainforest) estuaries.

Laboratory Analysis

Samples were sieved to <2.0 mm and homogenized for elemental and stable isotope analysis following Sartori et al. (2023). Ten mg of each sample were weighed in tin capsules for TN and $\delta^{15}\text{N}_{\text{total}}$, and in silver capsules (after decarbonation with $2 \text{ mol}\cdot\text{L}^{-1}$ HCl and drying at 110°C) for OC and $\delta^{13}\text{C}_{\text{org}}$. The analysis was performed using an elemental analyzer (Flash 2000) connected to a mass spectrometer (Delta V Advantage, Thermo Scientific) via a CONFLO IV interface. Quantification employed acetanilide standards, with $\sim 97\%$ inter-replicate precision. OC and TN contents were expressed as percentages (detection limits: 0.05% and 0.02% , respectively). $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{total}}$ values were expressed in ‰ relative to PDB and atmospheric N, with analytical precision of 0.1‰ .

For organic contaminants (PAHs, PCBs, PBDEs, and OCPs), extraction and analysis followed Santos et al. (2020). Briefly, 15 g of freeze-dried sediment were processed, and target compounds were identified and quantified by GC–MS/MS in MRM mode. Calibration was based on certified standards. Quantification limits (QLs) corresponded to the lowest calibration levels ($0.002 \text{ ng}\cdot\text{g}^{-1}$ for OCPs, $0.001 \text{ ng}\cdot\text{g}^{-1}$ for PCBs, and $0.006 \text{ ng}\cdot\text{g}^{-1}$ for PAHs), and concentrations below these limits were

excluded. QA/QC procedures included blanks (no interferences detected), surrogate recoveries (50–110%), spiked blanks and samples (50–110%), and certified reference material (IAEA-417), with measured concentrations within 60–90% (PAHs) and 75–90% (PCBs/OCPs) of certified values. The list of compounds analyzed and detailed information are provided in Table S2 and Figure S2.

To evaluate the sources for PAH compounds, the diagnostics ratios were plotted to detect combustion-derived PAH following Yunker et al. (2002) and Martins et al. (2010). The combined FI/FI+Py (FI: Fluoranthene; Py: Pyrene) and IP/IP+Bghi (IP: Indeno[123cd]pyrene; Bghi: Benzo[ghi]perylene) ratios help distinguish between petrogenic, petroleum combustion and biomass combustion: 0.2 – 0.4 indicates petroleum dominance; 0.4 – 0.5 petroleum combustion; and >0.5 sewage and/or biomass/coal combustion. Ratios of HCH isomers and DDT were used to evaluate historical pesticide use. PCB congeners were grouped according to IUPAC classification to differentiate regional atmospheric deposition from industrial or local sources.

Land Use Analysis

Land use and land cover data were obtained from the MapBiomas database (<https://plataforma.brasil.mapbiomas.org>), which provides annual land use maps for Brazil at 30 m spatial resolution from 1985 to 2024. Basin shapefiles were used to extract the area (km²) occupied by each land-use class for each year. The proportional area (%) of each category was then calculated relative to the total basin area (Tables S4 and S5). Full numerical precision is provided in the supplementary tables. Changes are expressed as percentage points relative to the total basin area.

Statistical Analysis

Linear regression models (lm function, base package, R Core Team, 2024) were used to assess temporal trends in land-use proportions (%) as a function of year for each basin. Additional linear models were applied to evaluate the relationship between TN and OC concentrations (%).

Differences between the basins were assessed by one-way ANOVA (aov, base package, R Core Team, 2024) or Kruskal-Wallis test for non-parametric (kruskal.test, base package, R Core Team, 2024). Two-way ANOVA was applied to test the effects of sampling environment (estuary and mangrove) and seasonality (and their interaction) within each basin. For non-parametric, the effects were evaluated using a

two-way approach based on the Aligned Rank Transform (ART) method (art, ARTool package, Kay et al., 2025), due to non-normality of residuals (Shapiro-Wilk test, $p < 0.05$). Post-hoc comparisons for significant interactions were performed using Tukey test (HSD.test, agricolae package, Mendiburu, 2023) and Dunn's test with Bonferroni adjustment (dunnTest, FSA package, Ogle et al., 2023; multcompLetters, multcompView package, Graves et al., 2024).

Isotopic biplot ($\delta^{13}\text{C}_{\text{org}}$ vs. $\delta^{15}\text{N}_{\text{total}}$ and $\delta^{13}\text{C}_{\text{org}}$ vs. $[\text{C:N}]_a$), were used to explore the distribution of sedimentary OM and to identify potential source endmembers. Endmember signatures were compiled from this study and previous literature. Marine OM signatures were defined as $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{total}}$ were -20.1 ± 0.8 and 5.3 ± 3.1 , respectively (Gatts et al., 2020; Serafim et al., 2023). Mangrove soil endmembers correspond to signatures measured in this study for both basin ($\delta^{13}\text{C}_{\text{org}} = -27.3 \pm 0.6$; $\delta^{15}\text{N}_{\text{total}} = 3.6 \pm 2.4$). For C_4 vegetation $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{total}}$ were -18.0 ± 0.7 and 4.6 ± 1.1 , respectively (Ribas, 2012). Sewage endmembers followed Vezzone et al. (2021), who reported isotopic signatures ($\delta^{13}\text{C}_{\text{org}} = -23.5 \pm 0.7$; $\delta^{15}\text{N}_{\text{total}} = 5.9 \pm 0.5$) from a lagoon chronically impacted by sewage discharge, where mixing model results indicated ~50% OM contribution from untreated wastewater. Discrimination factors for C_3 , C_4 and marine endmembers were retrieved from Serafim et al. (2023).

To estimate the relative contribution of potential OM sources to sedimentary pool, a Bayesian isotope mixing model was implemented (MixSIAR, MixSIAR package, Stock et al., 2018). Basin (CR and PSR) was included as a factor to estimate source contributions for each estuary separately. Convergence and model performance were assessed using standard diagnostics, including the Gelman–Rubin statistic, effective sample size, and visual inspection of trace plots and posterior density distributions. Source proportions were estimated using uniform Dirichlet priors ($\alpha = 1$). Model sensitivity to prior specification was evaluated by comparing results with weakly informative priors ($\alpha = 2, 1, 1, 2$). Posterior source contributions showed minimal variation between prior configurations, indicating that model outputs were primarily driven by isotopic data rather than prior assumptions.

Spearman's rank correlation (rcorr, Hmisc package, Harrell, 2024) and Principal Component Analysis (PCA, FactoMineR package, Sebastien et al., 2008) were used to evaluate the correlations among variables and to discriminate between the basins.

Correlations were considered moderate between 0.4 and 0.7 and strong above 0.7. All data were standardized using `decostand` function by `vegan` package for PCA analysis (Oksanen et al., 2024).

Models' assumptions were validated using diagnostic plots (Altman and Krzywinski, 2016), and when necessary, data were transformed to meet ANOVA premises (linearity, normality, homoscedasticity, leverage), using a maximum likelihood function (`boxcox`, MASS package, Venables and Ripley, 2002). All analyses were conducted at a 95% confidence level. All statistics and analyses were performed using R version 4.4.0 (R Core Team, 2024).

Results

Land-use change between 1985 and 2024

The MapBiomass data, in both basins, classify the land cover and use into: forest (i.e., forest formation, savanna formation, mangrove, floodable forest, wooded sandbank vegetation, wetland, grassland formation, hypersaline tidal flat, rocky outcrop and herbaceous sandbank vegetation), farming (i.e., pasture, agriculture, forest plantation, mosaic of uses), non-vegetated (i.e., beach, dune and sand spot, urban area, mining, other non-vegetated areas, photovoltaic power plant) and water (i.e., aquaculture, river, lake and ocean; Table S3).

Table 1 and Fig. S1 summarize the temporal dynamics of land use and land cover between 1985 and 2024 in the drainage basins of the CR (Amazon Rainforest) and the PSR basin (Atlantic Rainforest). In the CR basin, natural vegetation predominated throughout the period but declined from 93.1% in 1985 to 54.1% in 2024 (-39.0 percentage points), mainly due to farming expansion that increased 37.6 percentage points over the same period. In the PSR basin, anthropogenic land use remained dominant above 70%, with farming occupying 72.0% in 1985 and slightly declining to 68.2% in 2024 (-3.8 percentage points). Natural cover showed a modest increase of 2.6 percentage points, largely explained by forest recovery from 24.4% to 26.9% (+2.5 percentage points).

Table 1 Land use and land cover in the past 40 years in the hydrographic basins of the Caeté River (Amazon Rainforest) and the Paraíba do Sul River (Atlantic Rainforest).

Amazon Rainforest land use (%)	Year				
	1985	1995	2005	2015	2024
Forest	91.81	83.11	66.79	56.20	52.83
Herbaceous and shrubby vegetation	0.20	0.14	0.05	0.05	0.05
Farming	6.31	14.86	30.35	40.74	43.90
Non-vegetated área	0.58	0.81	1.47	1.67	1.99
Water	1.10	1.08	1.35	1.34	1.23
Natural	93.1	84.3	68.2	57.6	54.1
Anthropogenic	6.9	15.7	31.8	42.4	45.9
Atlantic Rainforest land use (%)					
Forest	24.44	24.26	24.70	26.09	26.89
Herbaceous and shrubby vegetation	1.11	0.86	1.12	1.13	1.05
Farming	71.99	71.91	70.84	69.25	68.16
Non-vegetated área	1.27	1.60	1.95	2.24	2.48
Water	1.19	1.13	1.13	1.05	1.20
Natural	26.8	26.5	27.2	28.6	29.4
Anthropogenic	73.2	73.5	72.8	71.4	70.6

Beyond the general contrast between natural and anthropogenic land use, subclass analysis highlights finer patterns. In the CR basin (Table S4), forest cover declined by 38.6 percentage points while pasture expanded by 35.8 percentage points, representing a shift from forest-dominated to pasture-dominated land use. The PSR basin (Table S5) forest cover showed a slight recovery of 2.5 percentage points, but pasture remained dominant (>48%) throughout the years, likely linked to reduced mosaic uses through improved landscape distinction. Additionally, forest plantations expanded, and urban areas more than doubled, underscoring the intensification and diversification of anthropogenic pressures. These patterns are consistent with the strong and significant temporal trend of increasing anthropogenic land use in the CR basin (slope = 1.175, $R^2 = 0.94$, $p < 0.0001$) and a slight but significant declining trend in the PSR basin (slope = -0.075 , $R^2 = 0.88$, $p < 0.0001$), indicating contrasting temporal trajectories of land use between basins.

Elemental and isotopic composition of organic matter

Fig. 2 illustrates the elemental and isotopic composition of surface sediment and mangrove soil from both estuarine zones, revealing distinct spatial and seasonal patterns between biomes, ecosystems, and hydrological periods.

Overall, PSR basin sediments showed higher OC concentration range compared to CR basin (from 0.02 to 5.4% [$1.3 \pm 1.5\%$], and from 0.04 to 1.3% [$0.6 \pm 0.6\%$], respectively), although the difference between the basins was not statistically significant (Fig. 2a; anova, $p > 0.05$). Seasonal variation was not significant when tested within each basin separately (Fig. 2b-c; $p > 0.05$). Differences were observed only between estuary and mangrove areas within basins (anova, $p < 0.0001$). In both basins, OC concentration was 3.9 and 4.7 times higher in mangroves areas compared to estuarine sediments for CR and PSR basins, respectively. TN concentration in the sediments from CR basin (from 0.03 to 3.5% [$0.7 \pm 1.1\%$]) was generally higher than PSR (from 0.02 to 0.6% [$0.3 \pm 0.2\%$]), but not statistically (Kruskal-Wallis, $p > 0.05$; Fig. 2d). In CR (Fig. 2e), TN concentration also showed a significant interaction between environment and season (anova, $p < 0.05$), with similarly high at mangrove and estuarine sites in the dry season, followed by a marked seasonal decline that was strongest at estuarine sites, where concentrations reached the lowest values during the rainy season. For PSR basin (Fig. 2f), only season had significant effects, with high values during the dry season (anova, $p < 0.05$). The (C:N)_a ratio was generally lower in the PSR basin (10.9 ± 5.6), but not statistically different (anova, $p > 0.05$) from CR basin (Fig. 2g; 15.0 ± 2.0). The (C:N)_a showed no statistical difference (anova, $p > 0.05$) in seasonality for both basins (~ 13.0). In contrast, across environments (mangrove soils and estuarine sediments), (C:N)_a values differed statistically, with higher values in mangrove soil (CR: 12.7 ± 1.8 and PSR: 17.8 ± 1.5) samples compared to estuarine sediments (CR: 15.0 ± 2.0 and PSR: 10.9 ± 5.5) for CR (Fig. 2h; anova, $p < 0.05$) and PSR (Fig. 2i; anova, $p < 0.001$) basin.

The sediment samples from CR basin were more ^{13}C -depleted ($-27.3 \pm 0.9\text{‰}$), compared with PSR basin ($-23.6 \pm 2.9\text{‰}$) (Kruskal-Wallis, $p < 0.01$; Fig. 2j). In the CR basin (Fig. 2k), $\delta^{13}\text{C}_{\text{org}}$ showed a significant interaction between environment and season (art, $p < 0.05$). Mangrove and estuary in dry season showed a significant ^{13}C -enrichment compared to both environment types in rainy season, but within season, environments did not show a significant difference. PSR basin (Fig. 2l), $\delta^{13}\text{C}_{\text{org}}$ also

showed a significant interaction between environment and season (art, $p < 0.05$), with estuarine sites consistently more ^{13}C -enriched than mangroves, regardless of the season. Moreover, $\delta^{15}\text{N}_{\text{total}}$ showed moderate enrichment ($5.6 \pm 1.0\text{‰}$; art, $p < 0.01$) in PSR basin compared with CR basin ($2.1 \pm 2.2\text{‰}$; Fig. 2m). $\delta^{15}\text{N}_{\text{total}}$ values were significantly (art, $p < 0.0001$) more ^{15}N -depleted during the dry season ($0.1 \pm 0.1\text{‰}$) compared to rainy season ($4.1 \pm 1.3\text{‰}$) in CR basin (Fig. 2n), but for PSR basin (Fig. 2o), no statistical difference was observed (art, $p > 0.05$).

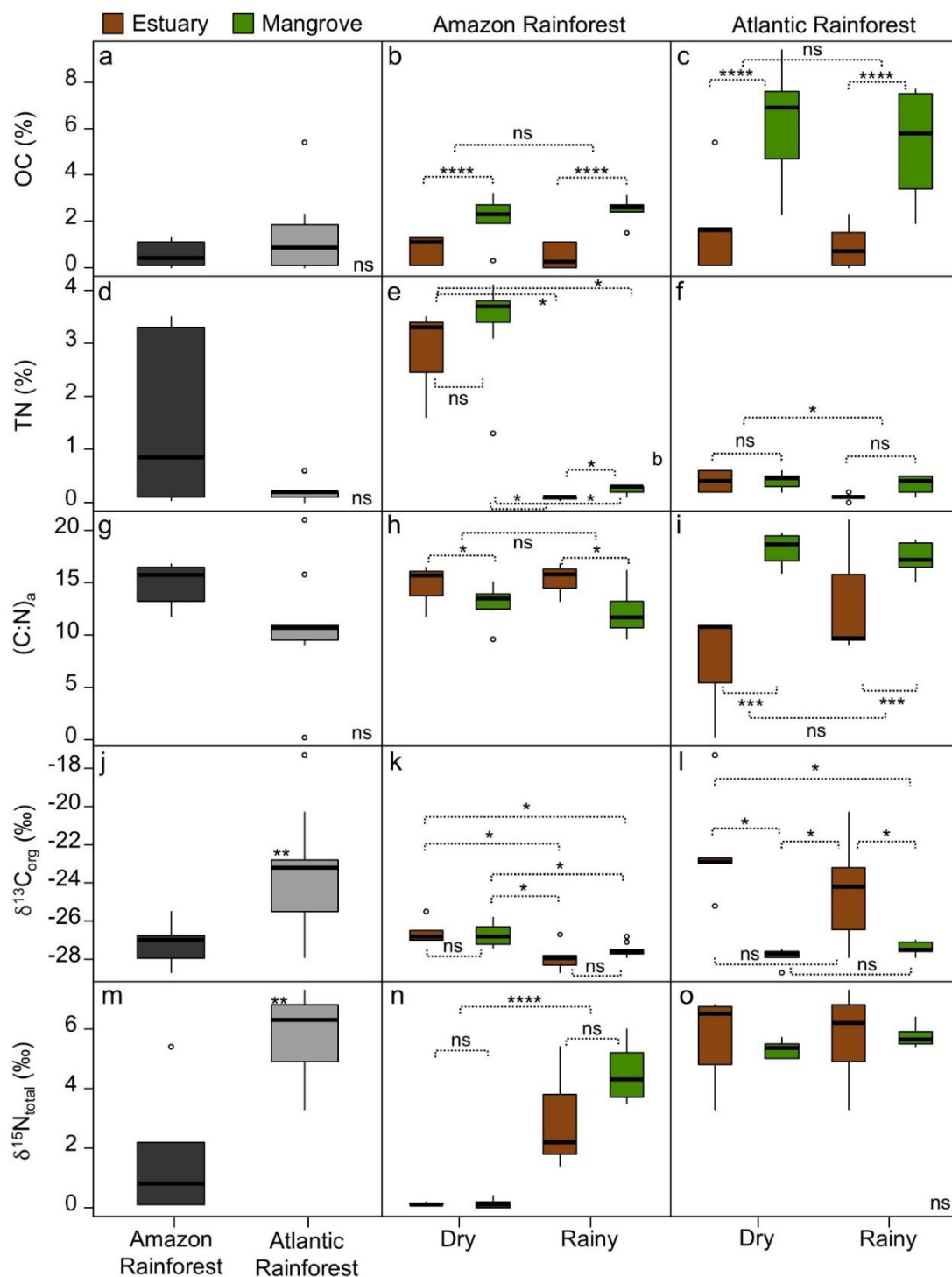


Fig. 2 Boxplots of OC and TN concentration (%), $\delta^{13}\text{C}_{\text{org}}$ (‰), $\delta^{15}\text{N}_{\text{total}}$ (‰), and (C:N)_a ratios in surface sediments and soils from estuarine and mangrove environments in the CR and PSR (Amazon and Atlantic Rainforests, respectively). Comparisons are shown by biome (1st column) and season (2nd and 3rd column). Significant differences are indicated above or below the boxplots with brackets: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant. When the interaction between factors is not significant, brackets represent only main effects. When the interaction is significant, multiple comparisons are performed among all factor combinations. Gray boxplots correspond to estuarine sediments only, as they reflect basin-scale changes.

The biplots of $\delta^{13}\text{C}_{\text{org}}$ vs. $\delta^{15}\text{N}_{\text{total}}$ (Fig. 3a) allowed the identification of potential OM sources. Most CR samples clustered within the $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{total}}$ ranges typical of C_3 mangrove vegetation, indicating a predominantly autochthonous contribution. Conversely, PSR sediments exhibit a broader isotopic dispersion, with several samples plotting between the mangrove and sewage endmembers, and some approaching the marine range, probably the samples located further east (lower longitudes), reflecting the increasing marine influence along the estuarine gradient toward the river mouth.

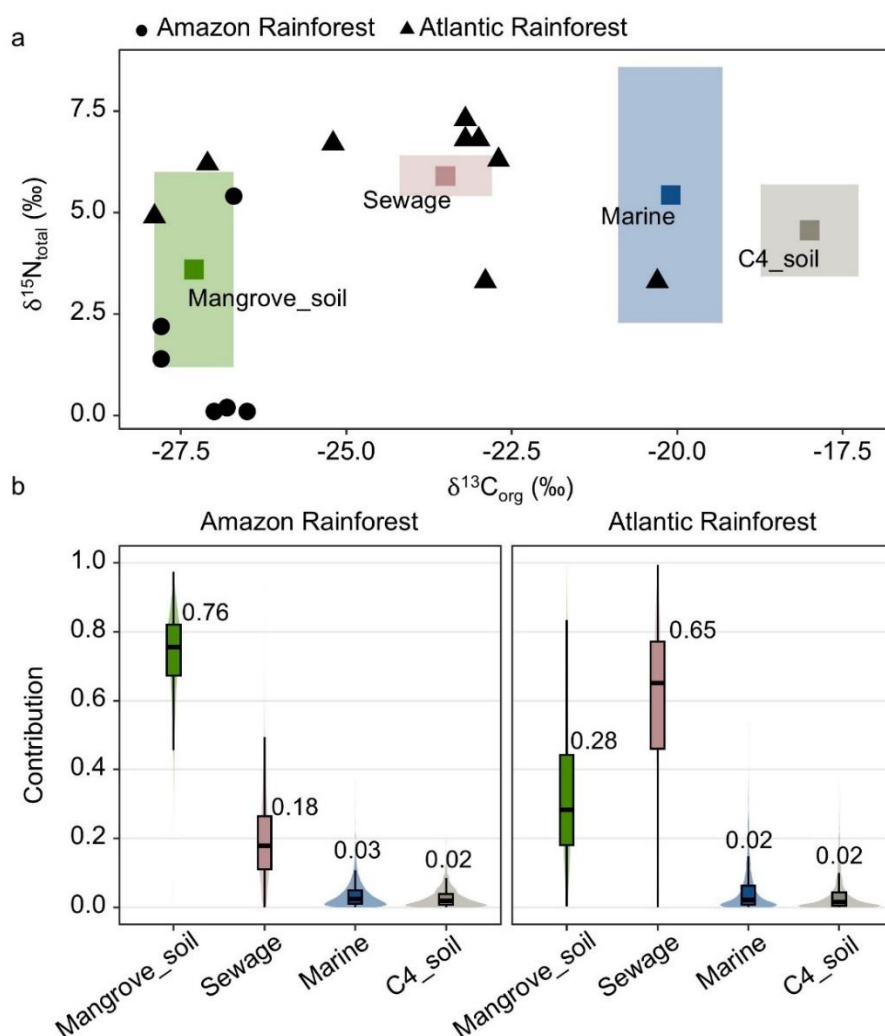


Fig. 3 Biplots of $\delta^{13}\text{C}_{\text{org}}$ (‰) vs. $\delta^{15}\text{N}_{\text{total}}$ (‰) (a) and posterior probability distributions of the proportional contribution of potential organic matter sources to sedimentary organic matter estimated using the Bayesian mixing model implemented in MixSIAR (b). Colored areas represent the mean and standard deviation of each end-member described in material and methods (e.g., C_3 and C_4 vegetation, sewage, marine sources). Violin plots represent the posterior density distributions of source contributions. The data were organized only with the results of $\delta^{13}\text{C}_{\text{org}}$ (‰) vs. $\delta^{15}\text{N}_{\text{total}}$ (‰).

The Bayesian mixing model indicated contrasting OM sources between the two estuarine systems. In the CR basin (Amazon Rainforest), mangrove-derived OM dominated sediment composition (median = 0.76), followed by sewage (0.18), while marine (0.03) and C4-derived OM (0.02) contributed minimally. In contrast, in the PSR basin (Atlantic Rainforest), sewage represented the dominant source (0.65), whereas mangrove showed a secondary contribution (0.28). Contributions from marine and C4 sources remained comparatively low in this basin (Fig. 3b). Although the PSR basin showed a tendency toward higher sewage contribution, the wide credible intervals of sewage and mangrove sources indicate and overlap, indicating an uncertainty in distinguishing these sources.

The relationship between TN and OC concentrations differed markedly between basins. In the PSR basin, TN increased linearly with OC ($R^2 = 0.95$, $p < 0.001$), indicating a strong covariation between both elements across the estuarine samples. In contrast, no significant relationship was observed in the CR basin ($R^2 = 0.04$, $p > 0.05$), suggesting a weaker coupling between TN and OC concentrations. These contrasting patterns indicate differences in the distribution and association of TN and OC between the two estuarine systems.

Organic pollutants distribution and sources

Among the analyzed compounds, several organochlorines and polybrominated substances exhibited concentrations below the analytical quantification limit (QL) of the method employed (Table S6). These include organochlorine pesticides (HCB, heptachlor and its epoxides, aldrin, isodrin, dieldrin, endrin, endosulfan I and II, methoxychlor, mirex, and chlordanes isomers) and PBDEs.

Correlation analysis for CR basin reveals that OC concentration is significantly positive moderate correlated with PAHs (sum of parental and alkylated PAHs), 16-PAHs, Alkyl-PAHs, high molecular weight (HMW)-PAHs, PCBs, DDTs and OCPs concentration ($r_s = 0.58, 0.53, 0.51, 0.54, 0.52, 0.47$ and 0.46 , respectively). They are also positive and moderate correlated with the DDT concentrations ($r_s = \sim 0.50$), except low molecular weight (LMW)-PAHs. PSR basin also showed significantly and positively moderate to higher correlation among OC concentration and PAHs, 16-PAHs, Alkyl-PAHs, LMW-PAHs, HMW-PAHs, PCBs and OCPs concentration ($r_s = 0.70, 0.68, 0.68, 0.65, 0.69, 0.54$ and 0.45 , respectively).

PCA results (Fig. S3) highlight the clear differences between the basins. PC1 explains 42% of the total variance and reflects industrial pollution and agricultural practices (PAHs, PCBs and DDTs) and is dominated by PSR estuarine samples. PC2 (20%) captures specifically agricultural contamination (OCPs, HCHs) and is associated with CR basin. Isotopic variables ($\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{total}}$) are aligned with PC1, showing enriched values in PSR and depleted values in CR, indicating that isotopic composition and contaminant concentrations co-vary between basins.

Fig. 4 shows the sum of each POP group in surface soil and sediment for CR and PSR basin. In general, CR basin ($53.1 \pm 48.6 \text{ ng}\cdot\text{g}^{-1}$) presents PAH concentrations 13.8 times lower (anova, $p < 0.0001$) than PSR basin ($736.4 \pm 880.3 \text{ ng}\cdot\text{g}^{-1}$; Fig. 4a). Fig. 4b exhibited a significant interaction between seasons and environment type in CR basin (anova, $p < 0.01$), with estuarine sediments during the rainy season presenting lower concentrations compared to sediments of dry season estuary and mangrove soils in both seasons. No differences were observed for PSR basin (Fig. 4c). Regarding HCH compounds, CR basin ($0.7 \pm 0.5 \text{ ng}\cdot\text{g}^{-1}$) showed concentrations more than 3 times higher (anova, $p < 0.0001$) compared PSR ($0.2 \pm 0.3 \text{ ng}\cdot\text{g}^{-1}$; Fig. 4d). Additionally, CR differed between environments and seasonality (Fig. 4e), with higher values in mangroves soils and dry season (anova, $p < 0.001$). In contrast, only seasonal effects were observed for PSR basin (anova, $p < 0.01$; Fig. 4f). As for DDT concentrations, PSR ($0.3 \pm 0.2 \text{ ng}\cdot\text{g}^{-1}$) was 1.7 times higher (anova, $p < 0.05$) than CR basin ($0.1 \pm 0.1 \text{ ng}\cdot\text{g}^{-1}$; Fig. 4g). No environmental or seasonal differences were observed within CR basin (Fig. 4h; anova, $p > 0.05$), while seasonal differences (anova, $p < 0.001$) were observed for PSR (Fig. 4i). For PCBs, PSR ($1.5 \pm 1.8 \text{ ng}\cdot\text{g}^{-1}$) presented concentrations more than 5 times higher (anova, $p < 0.0001$) compared to CR ($0.3 \pm 0.3 \text{ ng}\cdot\text{g}^{-1}$; Fig. 4j). Moreover, CR basin (Fig. 4k) statistically differs only between environments, with higher concentrations in mangrove soils (anova, $p < 0.01$). On the other hand, Fig. 4l showed significant interactions with only estuarine sites differing between seasonality (anova, $p < 0.01$).

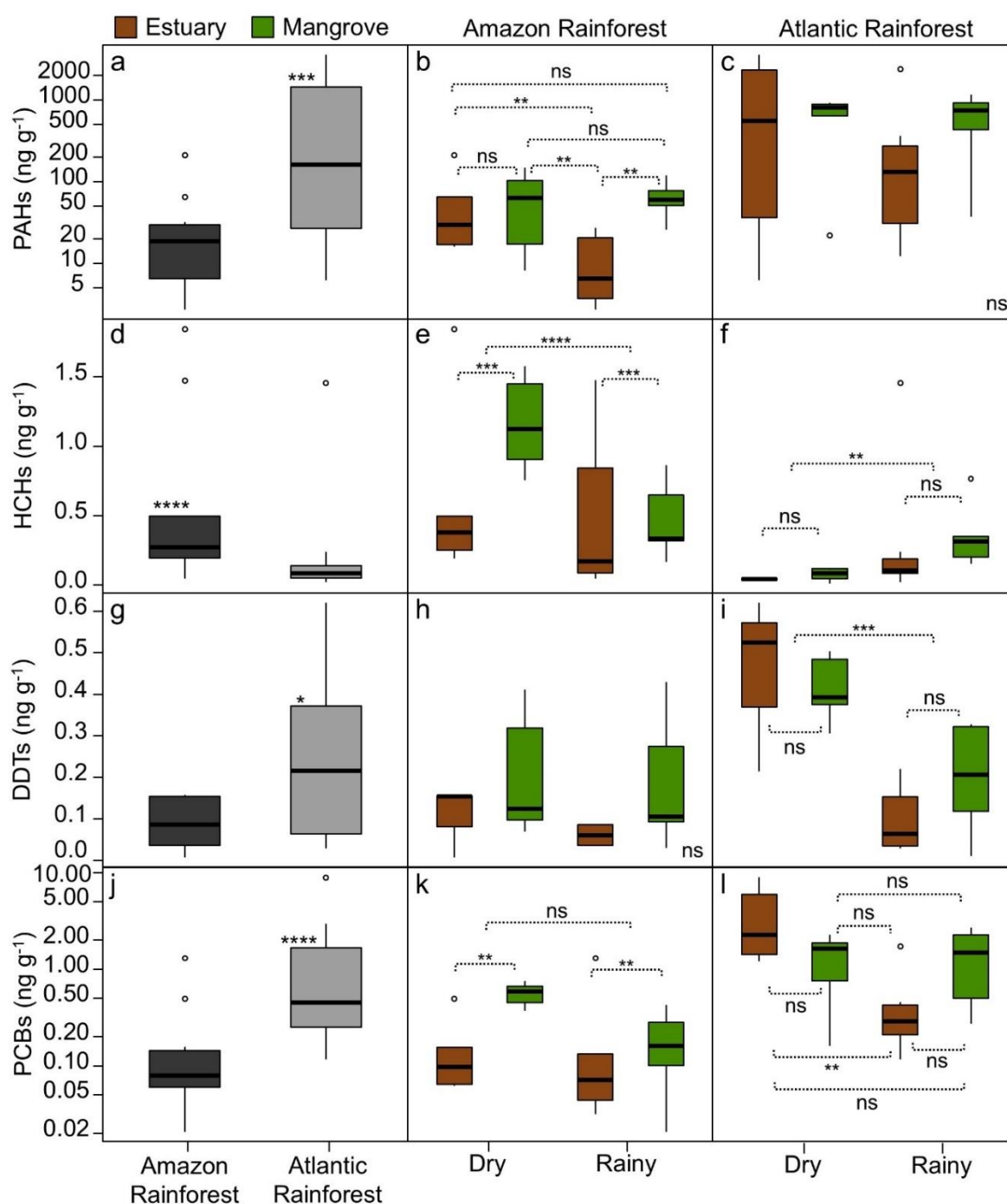


Fig. 4 Boxplots of PAHs, HCHs, DDTs and PCBs concentrations in surface sediments and soils from estuarine and mangrove environments in the CR and PSR (Amazon and Atlantic rainforest biomes, respectively). Comparisons are shown by biome (1st column) and season (2nd and 3rd column). Concentrations are given in ng g^{-1} . Significant differences are indicated above or below the boxplots with brackets: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns = not significant. When the interaction between factors is not significant, brackets represent only main effects. When the interaction is significant, multiple comparisons are performed among all factor combinations. A logarithmic scale was applied to the y-axis for PAHs and PCBs concentration for better visualization. Gray boxplots correspond to estuarine sediments only.

The diagnostic ratio between parent PAHs illustrates the distribution of samples according to their potential sources in both basins (Fig. 5). The results based on the IP/IP+Bghi and FI/FI+Py ratios indicate a predominance of pyrogenic sources, but with different sources contribution, ranging from petroleum to sewage and biomass/coal combustion (FI/FI+Py). While samples from the PSR basin showed particularly strong signatures of sewage and biomass combustion, samples from CR basin plot closer to the petroleum combustion and petrogenic sources, indicating mixed inputs with a stronger influence of fossil fuel-related sources. In addition, the spatial pattern of the samples supports distinct combustion sources between both basins, with elevated DDT concentrations co-occurring with biomass combustion sources, especially in the PSR basin, suggesting the overlap of agricultural pesticides use and burning practices or shared transport.

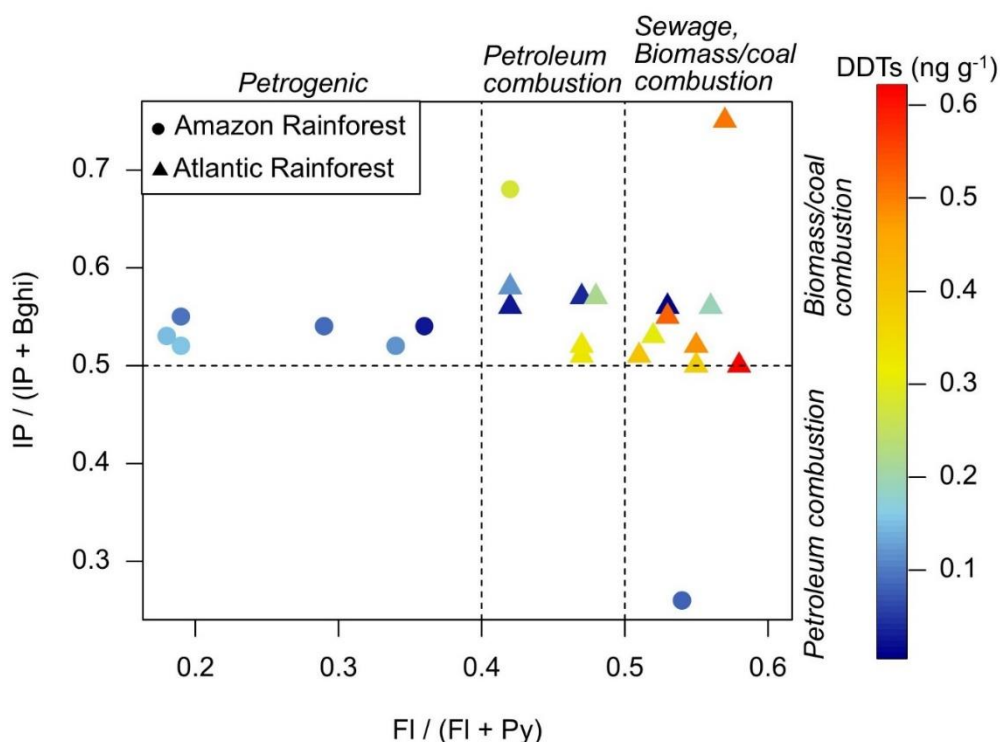


Fig. 5 PAH diagnostic ratios used to infer the sources of PAHs in the estuarine sediment samples from the Caeté River and Paraíba do Sul River colored by DDT. Many samples are not shown in the figure due to PAH concentrations below QL, preventing ratio calculations.

A relatively high proportion of β -HCH was observed in the CR basin (59% and 62% during dry and rainy seasons, respectively), whereas HCHs in the PSR basin consisted entirely of the γ -HCH isomer. The β -HCH/(α + γ -HCH) ratio in CR samples

was consistently above 0.5. Both basins showed mean (DDE+DDD)/DDT ratios above 0.50 (0.77 in CR and 0.85 in PSR), and DDD/DDE ratios lower than 1, except for one mangrove soil sample in each season. These ratios are commonly associated with aged residues and aerobic transformation processes reported in the literature.

The basins also exhibit distinct average distributions of PCB congener classes, with the PSR basin showing percentages up to two times higher in penta-, hexa-, and hepta-chlorinated PCB compared to the CR basin (24.6, 25.6, 13.3 and 14.5, 12.7, 0.25% respectively). This pattern is consistent with our PCA results (Table S6), which highlight the association of higher-chlorinated PCBs (penta, hexa and hepta-CB) with the PSR basin (PC1: 63% of the total variance), suggesting stronger contributions from local and industrial sources, in contrast to the CR basin, where lower-chlorinated congeners prevail (tri-CBs, PC2: 13% of the total variance), indicative of long-range atmospheric transport.

Discussion

Deforestation legacies and urban pressures

The PSR (Atlantic Rainforest) and CR (Amazon Rainforest) basins exhibit distinct trajectories in land-use dynamics, face different anthropogenic pressures and their mangrove ecosystems exhibit contrasting biogeochemical responses, especially in OM composition. These differences reflect their distinct histories of human occupation. Land-use change affects estuarine sediments through multiple interconnected pathways. Alterations in vegetation cover and agricultural expansion modify the quantity and composition of OM exported from drainage basins, which can be traced using stable isotopes. At the same time, different anthropogenic activities, such as urbanization, agriculture, fire management and fossil fuel combustion, release organic pollutants that are preferentially transported and preserved in sediments due to their strong affinity for OM (Gao et al., 2012; Bianchi et al., 2018). Taken together, the co-variation between isotopic composition and contaminant concentrations observed in the PCA (Fig. S3) indicates that these proxies respond to different processes occurring in the basins.

The Atlantic Rainforest, subject to land conversion since the 16th century (Dittmar et al., 2012; Solórzano et al., 2021; Pinto et al., 2023), has become Brazil's most industrialized and urbanized region. Within this biome, the PSR basin shows a

long-standing and stable anthropogenic alteration, with over 70% of its area under human use for at least four decades. The remaining forest fragments are largely restricted to protected areas (Pinto et al., 2023). Although anthropogenic areas have slightly declined in recent decades, their persistence reflects the enduring legacy of historical degradation. Current pressures include deforestation (Lämmle et al., 2022; Pinto et al., 2023), industrial effluents (Pfeiffer et al., 1986; Tonhá et al., 2021), untreated sewage (AGEVAP, 2020), and coastal urban expansion, all contributing to global mangrove loss (Lämmel et al., 2022; Pinto et al., 2023). A linear increase in non-vegetated areas is evident (Fig. S1), particularly in urban zones (Table S5).

The CR basin represents a recent phase of intense land-use change, though not yet marked by extensive anthropogenic expansion. Driven by government-led colonization policies since the 1970s (Gorayeb et al., 2009; 2011), this basin exhibits active landscape recovery dynamics, contrasting sharply with the long-term degradation legacies of the PSR basin. Between 1985 and 2024, pastures expanded from 6 to 41%, reflecting farming intensification in northeastern Pará (Raiol et al., 2019), where forests were converted into pasture (Table S4). Coastal mangroves, however, show notable resilience, safeguarded by RESEX creation in the early 2000s (Gorayeb et al., 2011; Abdala et al., 2012; Pereira et al., 2021). These contrasting land-use trajectories provide a consistent framework to interpret the coupled variations in OM composition and contaminant signatures observed between basins.

Stable isotopes reflect land-use legacies

Overall, the elemental and isotopic composition of OM from surface sediments capture these contrasting land-use settings, providing a geochemical fingerprint of past and ongoing human influence (Fig. 2a, d, g, j and m).

Surface sediments from the Amazon and Atlantic Rainforest estuaries exhibited clear contrasts in their $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}_{\text{total}}$ values, while OC concentration remained relatively similar between both biomes (Fig. 2). The Amazon estuary sediments were more ^{13}C -depleted, indicating the dominance of C_3 -plants as the main source. In contrast, the isotopic enrichment and clustering of PSR sediments near sewage, marine, and C_4 -vegetation endmembers indicate a mixture of anthropogenic and natural inputs shaping the estuarine OM pool (Prado et al., 2020; Serafim et al., 2023), consistent with the higher contaminant concentrations observed in this basin. Earlier

studies in the Amazon basin reported $\delta^{13}\text{C}_{\text{org}}$ values between -27.5 and -25.8‰ for riverine sediments (Bouchez et al., 2014), consistent with C_3 -vegetation, while a broader range, from -28.4 to -17.5‰, was found in the estuarine zone reflecting a mixture of terrestrial and marine sources (Cai et al., 1988). More detailed spatial assessments show that the most ^{13}C -depleted values (-31 to -27‰) occur in the inner estuary, while values gradually increase toward the estuary mouth, reaching approximately -25‰ (Weirich et al., 2025). More recently, Serafim et al. (2023) compared $\delta^{13}\text{C}_{\text{org}}$ values of coastal sediments from both biomes and found ^{13}C -enriched values for the Atlantic Rainforest ($-22.6 \pm 1.3\text{‰}$) compared to the Amazon Rainforest ($-25.0 \pm 3.1\text{‰}$) coastal sediments, with C_3 -plants contributing to 35.6 and 51.3% to the OC concentration from the areas, respectively. Lower (C:N)_a ratios in Atlantic Rainforest sediments further indicate greater contribution from primary producers and/or N-enriched anthropogenic sources, while higher ratios in the Amazon reflect more vascular plant-derived material. Overall, these isotopic patterns capture the stronger historical and ongoing anthropogenic imprint on the Atlantic Rainforest basin, while the Amazon system still retains a largely natural, vegetation-dominated composition.

Within the Amazon Rainforest, the CR basin, macrotidal dynamics strongly modulate the composition of OM (Fig. 2b, e, h, k and n; Fig. 3). While the elemental composition does not change over season, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ varies. During the dry season, low river discharge enhances tidal exchange and marine intrusion, with mangrove flats acting predominantly as sediment importers (Asp et al., 2018). This hydrodynamic setting increases the relative marine contribution, leading to comparatively more ^{13}C -enriched and ^{15}N -depleted signatures in estuarine sediments and mangrove soils. Conversely, during the rainy season, mangrove OM export is intensified, with both dissolved and particulate fractions being exported in similar proportions (Dittmar et al., 2001). This enhanced export, together with increased surface runoff, results in more ^{13}C -depleted and ^{15}N -enriched values, consistent with anthropogenic inputs such as sewage discharge (Ribas, 2012; Prado et al., 2020; Vezzzone et al., 2021; Park et al., 2023). Effluent discharges reported by Monteiro et al. (2016) have shown that the CR estuary is affected, emphasizing sewage as an additional OM source. Despite upstream anthropogenic disturbances, the mangrove area has remained relatively stable over the last four decades (McLachlan et al., 2020; Table S4), displaying ^{13}C -depleted values controlled by the seasonality rather than

progressive degradation. This pattern is further supported by the mixing model results, which indicate dominance of mangrove-derived OM in CR and sewage-derived OM in PSR.

The lack of relationship between TN and OC concentrations in the CR basin ($R^2 = 0.043$, $p > 0.05$) points to a biogeochemical decoupling potentially driven by post-depositional alteration and selective degradation (Gao et al., 2012; Guo et al., 2020). In such dynamic environments, nitrogen typically undergoes more rapid mineralization than carbon, leading to a loss of original biogenic signatures and a convergence of C/N ratios that may mask source identification. This decoupling might be also mediated by granulometric control, where the sediment texture governs the physical protection and preservation of OM (Craig, 1953; Thornton and McManus, 1994; Keil and Mayer, 2014; Bianchi et al., 2018). These findings align with the intense OM remineralization processes reported for the Amazon River plume by Serafim et al. (2023), reinforcing the role of mangroves as biogeochemical filters that attenuate upstream anthropogenic signals before they reach the ocean. In contrast, the PSR estuary, characterized by a microtidal regime, is more strongly influenced by river discharge (Shi et al., 2023). This hydrodynamic setting, combined with long-term land-use transformation, governs the isotopic and elemental composition of surface sediments in the PSR basin. The strong linear relationship between TN and OC ($R^2 = 0.95$) is consistent with a conservative mixing regime of refractory OM from distinct terrestrial and marine endmembers. This coupling suggests that distributions of TN and OC in the PSR basin are primarily governed by hydrodynamic transport and continental runoff, reflecting a pool dominated by organic nitrogen with limited contribution from mineral-bound nitrogen (Thornton and McManus, 1994; Keil and Mayer, 2014; Sartori et al., 2023).

In the Atlantic Rainforest, the PSR basin shows that spatial variability rather than seasonality drives the composition of OM in the estuarine sediment (Fig. 2c, d, i and l; Fig. 3). This lack of seasonality is likely explained by the decrease in runoff rates due to decades of human activities upstream and the decrease in river flow and precipitation rates, intensifying drought conditions during the dry season and reducing rainfall even in the rainy season (Oliveira et al., 2023). Distinct isotopic values between the mangrove and estuarine sites revealed contrasting OM composition: mangrove soils presented ^{13}C -depleted and ^{15}N -enriched values, reflecting their own contribution, while estuarine sediments had shown a different pattern, indicating a greater marine influence and likely reflecting the changes in the drainage basin. This spatial gradient

highlights the efficient mixing and transformation of OM along the land-ocean continuum, controlled by geomorphology and limited tidal exchange. These findings are consistent with other southeastern Brazilian estuary under moderate hydrodynamic exchange and anthropogenic influence (Brandini et al., 2022). Nevertheless, it is important to note the wider range of $\delta^{13}\text{C}_{\text{org}}$ values observed during the rainy season (Fig. 2i). The more ^{13}C -depleted signatures likely reflect a greater contribution of historical C_3 -plant material leached from deeper soils, combined with inputs from mangrove soils, as previously reported by Marques et al. (2017) and Serafim et al. (2023) for dissolved OM and coastal sediments, respectively.

Previous studies (e.g., Marques et al., 2017; Lizaga et al., 2021; Serafim et al., 2023, Dow et al., 2024) have shown that historical land-cover changes and continuous anthropogenic pressure have left a lasting isotopic legacy in estuarine sediments, often overriding natural sorting processes. Together, the isotopic and elemental composition patterns across both basins emphasize the diagnostic power of stable isotopes to disentangle natural and anthropogenic signals in tropical estuarine systems. While the estuarine sediments in the CR basin still reflect a system under dynamic yet partially buffered conditions, where mangrove productivity and tidal exchange mitigate the effects of land-use change upstream, in the PSR basin, reflects a higher persistent anthropogenic imprint, marked specially by $\delta^{13}\text{C}_{\text{org}}$ values as a result to the long-term anthropogenic pressure as well agricultural inputs.

Fingerprints of anthropogenic land-use through POPs

Extending the perspective on molecular markers of anthropogenic pressure, the distribution of POPs can further illustrate how human activities are recorded in tropical estuarine sediments. While OM tracers integrate the combined effects of vegetation type, OM degradation, and hydrodynamic regime, POPs provide a complementary and more direct signal of industrial, agricultural, and urban emissions associated with land-use history. Since most POPs are hydrophobic, their environmental behavior is mostly shown to be tightly coupled to the transport and burial of OM (Hu et al., 2010).

Estuarine surface sediments from the Amazon and Atlantic Rainforests showed clear contrasts in POP concentrations (Fig. 4a, d, g, j), reflecting the distinct land-use trajectories of their drainage basins (Table 1). In the PSR estuary, PAH, DDT and PCB concentrations were markedly higher than in the CR estuary, consistent with the long-

term legacy of land conversion, industrialization and urbanization. PAH concentrations in PSR ($<QL - 2808.28 \text{ ng}\cdot\text{g}^{-1}$, 16-PAHs) exceeded values reported for several tropical estuaries, including the Subarnarekha (India, $181.2 - 511.3 \text{ ng}\cdot\text{g}^{-1}$; Ambade et al., 2021), Yellow River (China, $31.0 - 133.0 \text{ ng}\cdot\text{g}^{-1}$; Li et al., 2006), and surface sediments from Todos os Santos Bay (Brazil, $11.59 - 1825.35 \text{ ng}\cdot\text{g}^{-1}$; Nascimento et al., 2017), placing PSR among the most contaminated tropical estuarine systems. PCB concentrations were also higher in PSR ($<DL - 8.94 \text{ ng}\cdot\text{g}^{-1}$), consistent with enrichment typically observed near industrial and urban centers (up to $4.78 \text{ ng}\cdot\text{g}^{-1}$; Souza et al., 2018a). Higher values have also been documented in other tropical estuaries in Brazil, such as the Santos estuary, with PCBs concentrations reaching $190.7 \text{ ng}\cdot\text{g}^{-1}$ (Souza et al., 2018b).

In contrast, the CR estuary exhibited overall lower POP loads, but a contamination pattern dominated by OCPs. HCH concentrations were higher in the CR estuarine sediments ($< QL - 1.84 \text{ ng}\cdot\text{g}^{-1}$) compared to PSR estuary ($< QL - 1.43 \text{ ng}\cdot\text{g}^{-1}$), consistent with a stronger agricultural influence. This pattern aligns with the ongoing land-use change in CR basin, where rapid agricultural expansion (from 6.31 to 43.90%, from 1985 to 2024; Table 1) and limited regulatory control have shaped a contamination profile dominated by pesticides rather than industrial or combustion-derived pollutants. These levels are comparable to those reported for the Paranaguá Estuarine System (Brazil, $< DL - 1.68 \text{ ng}\cdot\text{g}^{-1}$; Souza et al., 2018a). DDT concentrations in both estuaries ($<QL - 0.43$ in CR; $<QL - 0.62 \text{ ng}\cdot\text{g}^{-1}$ in PSR) were lower than those reported for Paranaguá (Brazil, $<DL - 3.22 \text{ ng}\cdot\text{g}^{-1}$; Souza et al., 2018a). Overall, the observed concentration ranges indicate that POP accumulation in PSR aligns with the upper end reported for tropical estuaries due to persistent industrial and urban influence, while CR basin reflects an agricultural-driven profile marked primarily by HCH contamination.

The predominance of HMW-PAHs in PSR basin may indicate fossil fuel combustion linked to urban centers and vehicular emissions (Maioli et al., 2011). Beyond these inputs, the dominance of combustion-derived PAHs in our samples (Figure 4), along with depleted $\delta^{13}\text{C}_{\text{org}}$ values (Fig. 2) and the documented use of agricultural fire management practices over the past four decades (Table 1), suggest a strong influence of both petroleum and biomass burning on sedimentary OM. Additionally, Fig. 5 reveals signal consistent with pyrogenic sources, although some diagnostic ratios (e.g., $\text{Fl}/(\text{Fl}+\text{Py})$) can also reflect sewage/urban effluent inputs. Therefore, we interpret these signals as indicative of mixed combustion and/or

sewage-related inputs rather than exclusively pyrogenic sources (Martins et al., 2010). Previous analyses of black carbon (BC), another proxy for biomass and fossil fuel incomplete combustion showed that historical burning of the Atlantic Rainforest and agricultural practices substantially increase the deposition of combustion products, which are transported from terrestrial systems to coastal environments along with the bulk OM (Dittmar et al., 2012; Marques et al., 2017; Serafim et al., 2023). In addition, the marked variability in DDT concentrations in the PSR indicates the influence of agricultural and pasture-management practices, such as pesticide application and biomass burning, which modulate the molecular markers observed in the system. Distinctly, although the CR estuary presents an overall agricultural contamination profile, its PAH signatures indicate mixed combustion sources, with samples ranging from petrogenic to pyrogenic inputs (Fig. 5), likely reflecting contributions from localized seasonal field fire, small-scale boat traffic and fuel-oil spills (Yunker et al., 2002; Gorayeb et al., 2009).

HCH and DDT can also enter aquatic systems through surface runoff and sorption to particles (Parween et al., 2014). These OCPs were widely used in Brazilian agriculture and as a disease vector control from the 1940s until their complete ban in 2009, making Brazil a notable case on the global stage (Oliveira et al., 2016; Fernandes et al., 2020). Our results support this historical pattern in CR basin due to high proportion of β -HCH (~60%), and β -HCH/(α + γ -HCH) ratio above 0.5. β -HCH is the most stable, persistent and resistant isomer among the HCH (Yi et al., 2013). With no recent input of technical HCH, the α -HCH and γ -HCH can be converted into β -HCH, increasing its concentration (Yang et al., 2010). Besides, technical HCH present β -HCH concentration around 5–14% (Hu et al., 2010), thus, the enrichment to 60% can also indicate degradation of the other isomers (Hu et al., 2010; Tang et al., 2020). The predominance of β -HCH in the CR basin is consistent with the historical use of technical HCH, a low-cost formulation widely applied in Brazil, particularly in socioeconomically vulnerable regions such as Pará State, where limited access to more expensive lindane (γ -HCH) and weak regulatory enforcement likely favored its use (Gorayeb et al., 2009; Oliveira et al., 2016). PSR basin on the other hand, show only γ -HCH concentration, which is associated with lindane use (Yi et al., 2013). Regarding the DDT class of compounds, the DDE+DDD/DDT ratios for both basins far exceeded the threshold of 0.5, indicating that the detected residues primarily originate

from past applications rather than recent inputs (Yang et al., 2010; Sreedevi et al., 2023). The predominance of DDE over DDD indicates that DDT residues have mainly undergone aerobic biodegradation (Oliveira et al., 2016). Such conditions are likely favored by strong tidal currents and sediment reworking, which enhances oxygen penetration and promotes the persistence of DDE as the dominant metabolite.

PCBs are unintentionally formed and released during incomplete combustion of materials that contain chlorine; however, they are primarily associated with industrial activities and electrical applications (Nam et al., 2008; Mao et al., 2021). The observed differences in PCB concentration and congener distributions between the PSR and CR basins highlight these contrasting contamination patterns (Table S6). The PSR basin exhibits notably higher proportions and association with heavier PCBs suggesting a predominance of local/industrial sources (Mao et al., 2021), while the CR basin is dominated by lower-chlorinated congeners (tri-CBs), indicating inputs likely associated with long-range atmospheric transport (Nam et al., 2008; Mao et al., 2021). Such patterns reinforce the distinct anthropogenic signatures of each basin and the role of hydrodynamics in regulating contaminant deposition and export.

Hydrodynamic setting further modulates POPs behavior. Macrotidal dynamics in CR can promote resuspension and dilution, enhancing contaminant export to adjacent shelves, while the microtidal PSR estuary favors fine-sediment accumulation and long-term pollutant retention. The CR and PSR basins show the tendency to present lower concentrations for all POPs in estuarine sediments during the rainy season. However, no seasonal or spatial differences were observed for PAHs in the PSR basin, and DDTs and PCBs in CR basin suggesting a continuous input of mixed combustion sources, pesticides byproducts and atmospheric deposition, respectively. This pattern may be associated with persistent emissions from fossil fuel combustion, particularly in urban and industrial areas, as well as biomass burning linked to agricultural practices for PSR and pest control in agricultural, household and boat paint for CR basin (Maioli et al., 2011; Oliveira et al., 2016; Tang et al., 2020; Gurgatz et al., 2023). These anthropogenic molecular markers capture persistent inputs from combustion, agriculture, and industrial sources, reflecting distinct land-use legacies in the CR and PSR basins. Despite their relevance, such integrative approaches remain scarce in Brazilian estuarine research, underscoring the contribution of this study to understanding human impacts in tropical coastal environments.

Overall, the combined analysis of stable isotopes, organic contaminants, and land-use trajectories demonstrates that these systems exhibit coherent and coupled responses, where OM sources, contaminant inputs, and transport processes are mutually controlled by watershed-scale anthropogenic activities.

Conclusion

Our study highlights a clear contrast in the biogeochemical responses of Amazonia (CR) and Atlantic (PSR) Rainforest estuarine-mangrove systems under anthropogenic pressures. The CR basin, despite recent land-use changes, shows resilient mangroves attenuate upstream inputs, whereas the PSR basin reflects profound alteration from historical and ongoing human impacts. Stable isotopes and elemental ratios reveal contrasting sources and transformations of OM between the basins, while the distribution and composition of POPs provide complementary evidence of the dominant anthropogenic activities influencing each system. The co-occurrence of isotopic signatures and contaminant patterns, supported by multivariate analysis, demonstrates that these proxies respond to common watershed-scale processes linked to land-use trajectories. When interpreted, these proxies indicate that persistent pressures in the PSR basin, associated with intensive land use and urban emissions, override natural estuarine processes and produce a distinct geochemical imprint in surface sediments. In contrast, the CR basin, where mangroves remain largely preserved despite farming expansion, still exhibits OM signatures dominated by C₃-derived inputs and comparatively lower contamination levels. POP signatures further distinguish these anthropogenic drivers: PAH ratios confirm pyrogenic sources in both basins, with PSR influenced by sewage/biomass burning, and fossil fuels, and CR with mixed combustion. PSR samples also show lindane and heavy PCB congeners from industrial activities, whereas CR sediments reflect historical HCH use and lighter PCB congeners consistent with diffuse atmospheric transport.

Together, these results indicate that OM composition and contaminant accumulation, are coupled and controlled not only by hydrodynamic conditions, but also reflects land-use history across basins. These findings underscore contrasting pollution patterns and the need for region-specific management. Overall, the combined use of isotopic tracers, molecular markers, and land-use reconstruction provides a coherent framework for understanding how different histories of human occupation

shape OM dynamics and pollutant accumulation in tropical estuarine systems, reinforces mangroves as biogeochemical barriers and informs future studies on human impacts in coastal systems.

CRedit authorship contribution statement

Échily Sartori: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization.

Cristiane dos Santos Vergilio: Conceptualization, Writing – review & editing, Supervision.

Tassiana S. G. Serafim: Formal analysis, Writing – review & editing, Visualization.

Diego Larcerda: Formal analysis, Writing – review & editing.

Luiza Silva do Nascimento: Investigation.

Pedro Vianna Gatts: Investigation, Writing – review & editing.

Mariana Freitas: Investigation, Writing – review & editing.

Jamile Tamer Nasser: Methodology, Validation, Data curation.

Marcelo Gomes de Almeida: Methodology, Validation, Writing – review & editing.

César de C. Martins: Validation, Writing – review & editing, Resources.

Nils E. Asp: Investigation, Writing – review & editing.

Carlos Eduardo de Rezende: Conceptualization, Validation, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgments

We thank the Environmental Science Laboratory (Laboratório de Ciências Ambientais – LCA) of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, the Marine Organic Chemistry Laboratory of the Universidade de São Paulo and the Coastal Geology Laboratory of the Universidade Federal do Pará, for providing infrastructure for sampling collection and further analysis. The final stage of this study was carried out with the participation of Carlos E De Rezende during his period as a fellow at Hanse-Wissenschaftskolleg – Institute for Advanced Study (HWK).

Funding statement

É. Sartori received grant from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (88887.824229/2023-00) - Finance Code 001. É. Sartori and L. S. do Nascimento received scholarship from the CAPES/DAAD Probral Program, which

supports international academic cooperation between Brazil and Germany (88887.989816/2024-00 and 88887.985811/2024-00). C.C. Martins thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant code 310850/2020-7). Carlos E. de Rezende received grant from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; Grant Code E-26/203.950/2024) and CNPq (Grant Code 310.260/2023-0 and 409.378/2022-4).

Declaration of Interest Statement

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.

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

All the text of the manuscript was initially written without AI. AI was used to correct some English formulations.

Data availability

Data will be made available on request.

References

- Abdala, G., Saraiva, N., & Wesley, F., 2012. *Plano de Manejo da Reserva Extrativista Caeté-Taperaçu - VOLUME I - Diagnóstico da Unidade de Conservação* (pp. 0–109). ICMBio
- AGEVAP - Associação Pro-Gestão das Águas da Bacia Hidrográfica do Rio Paraíba do Sul., 2020. Relatório sobre cenário ambiental 2020. 171p. <https://www.ceivap.org.br/>
- Altman, N., & Krzywinski, M., 2016. Regression diagnostics. *Nature Methods*, 13(5), 385–387. <https://doi.org/10.1038/nmeth.385>
- Álvarez, S., & Valpassos, C. A. M. (Eds.). (2023). Alguns olhares do Sul: Antropologia, etnografia, e análise de conflitos e crises no século XXI. EDUENF, 95-103.
- Ambade, B., Sethi, S. S., Giri, B., Biswas, J. K., & Baudh, K., 2021. Characterization, Behavior, and Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) in

- the Estuary Sediments. *Bulletin of Environmental Contamination and Toxicology*, 108(2), 243–252. <https://doi.org/10.1007/s00128-021-03393-3>
- ANA., 2011. *Estudos auxiliares para a gestão do risco de inundações. Bacia do Rio Paraíba do Sul - Setor Policial*. Agência Nacional de Águas e Saneamento Básico.
- Asp, N. E., Gomes, V. J. C., Schettini, C. A. F., Souza-Filho, P. W. M., Siegle, E., Ogston, A. S., Nittrouer, C. A., Silva, J. N. S., Nascimento, W. R., Souza, S. R., Pereira, L. C. C., & Queiroz, M. C., 2018. Sediment dynamics of a tropical tide-dominated estuary: Turbidity maximum, mangroves and the role of the Amazon River sediment load. *Estuarine, Coastal and Shelf Science*, 214, 10–24. <https://doi.org/10.1016/j.ecss.2018.09.004>
- Bernardes, M. C., Martinelli, L. A., Krusche, A. V., Gudeman, J., Moreira, M., Victoria, R. L., Ometto, J. P. H. B., Ballester, M. V. R., Aufdenkampe, A. K., Richey, J. E., Hedges, J. I., 2004. Riverine organic matter composition as a function of land use changes, southwest Amazon. *Ecol. Appl.* 14, 263–279. doi: 10.1890/01-6028
- Bianchi, T.S., Cui, X., Blair, N.E., Burdige, D.J., Eglinton, T.I., Galy, V., 2018. Centers of organic carbon burial and oxidation at the land-ocean interface. *Organic Geochemistry* 115, 138–155. <https://doi.org/10.1016/j.orggeochem.2017.09.008>
- Bouchez, J., Galy, V., Hilton, R. G., Gaillardet, J., Moreira-Turcq, P., Pérez, M. A., France-Lanord, C., & Maurice, L., 2014. Source, transport and fluxes of Amazon River particulate organic carbon: Insights from river sediment depth-profiles. *Geochimica et Cosmochimica Acta*, 133, 280–298. <https://doi.org/10.1016/j.gca.2014.02.032>
- Bouillon, S., Borges, A.V., Castañeda-Moya, E., Diele, K., Dittmar, T., Duke, N.C., Kristensen, E., Lee, S.Y., Marchand, C., Middelburg, J.J., Rivera-Monroy, V.H., Smith, T.J., III, Twilley, R.R., 2008. Mangrove production and carbon sinks: A revision of global budget estimates. *Global Biogeochemical Cycles* 22. <https://doi.org/10.1029/2007gb003052>
- Bouillon, S., Connolly, R. M., & Gillikin, D. P., 2011. Use of Stable Isotopes to Understand Food Webs and Ecosystem Functioning in Estuaries. In *Treatise on Estuarine and Coastal Science* (Vol. 7, pp. 143–173). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-374711-2.00711-7>

- Brandini, N., da Costa Machado, E., Sanders, C. J., Cotovicz, L. C., Jr., Bernardes, M. C., & Knoppers, B. A., 2022. Organic matter processing through an estuarine system: Evidence from stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and molecular (lignin phenols) signatures. *Estuarine, Coastal and Shelf Science*, 265, 107707. <https://doi.org/10.1016/j.ecss.2021.107707>
- Broggio, I.S., Silva-Junior, C.H.L., Nascimento, M.T., Villela, D.M., Aragão, L.E.O.C., 2024. Quantifying landscape fragmentation and forest carbon dynamics over 35 years in the Brazilian Atlantic Forest. *Environ. Res. Lett.* 19, 034047. <https://doi.org/10.1088/1748-9326/ad281c>
- Bunting, P., Rosenqvist, A., Hilarides, L., Lucas, R.M., Thomas, N., Tadono, T., Worthington, T.A., Spalding, M., Murray, N.J., Rebelo, L.-M., 2022. Global mangrove extent change 1996–2020: global mangrove watch version 3.0. *Remote Sens* 14, 3657. <https://doi.org/10.3390/rs14153657>.
- Cai, D.-L., Tan, F.C., Edmond, J.M., 1988. Sources and transport of particulate organic carbon in the Amazon River and estuary. *Estuar. Coast. Shelf Sci.* [https://doi.org/10.1016/0272-7714\(88\)90008-x](https://doi.org/10.1016/0272-7714(88)90008-x).
- Cornelissen, G., Gustafsson, Ö., Bucheli, T. D., Jonker, M. T. O., Koelmans, A. A., & van Noort, P. C. M. (2005). Extensive Sorption of Organic Compounds to Black Carbon, Coal, and Kerogen in Sediments and Soils: Mechanisms and Consequences for Distribution, Bioaccumulation, and Biodegradation. *Environmental Science & Technology*, 39(18), 6881–6895. <https://doi.org/10.1021/es050191b>
- Cotovicz, L. C., Vidal, L. O., de Rezende, C. E., Bernardes, M. C., Knoppers, B. A., Sobrinho, R. L., Cardoso, R. P., Muniz, M., dos Anjos, R. M., Biehler, A., & Abril, G., 2020. Carbon dioxide sources and sinks in the delta of the Paraíba do Sul River (Southeastern Brazil) modulated by carbonate thermodynamics, gas exchange and ecosystem metabolism during estuarine mixin. *Marine Chemistry*, 226. <https://doi.org/10.1016/j.marchem.2020.103869>
- Craig, H. (1953). The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, 3(2–3), 53–92. [https://doi.org/10.1016/0016-7037\(53\)90001-5](https://doi.org/10.1016/0016-7037(53)90001-5)
- Dittmar, T., de Rezende, C. E., Manecki, M., Niggemann, J., Coelho Ovalle, A. R., Stubbins, A., & Bernardes, M. C., 2012. Continuous flux of dissolved black

- carbon from a vanished tropical forest biome. *Nature Geoscience*, 5(9), 618–622. <https://doi.org/10.1038/ngeo1541>
- Dittmar, T., Lara, R. J., & Kattner, G., 2001. River or mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. In *Marine Chemistry* (Vol. 73). [https://doi.org/10.1016/S0304-4203\(00\)00110-9](https://doi.org/10.1016/S0304-4203(00)00110-9)
- Dow, S., Ouimet, W. B., & Hren, M. T., 2024. Legacy impacts and recovery of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and C/N storage in soils due to historic land use. *Anthropocene*, 46. <https://doi.org/10.1016/j.ancene.2024.100435>
- Eisma, D., Chen, S., Li, A., 1994. Tidal variations in suspended matter floc size in the Elbe river and Dollard estuaries. *Netherlands Journal of Aquatic Ecology* 28, 267–274. <https://doi.org/10.1007/bf02334194>
- Fernandes, C. L. F., Ramires, P. F., De Moura, R. R., Pohren, R. de S., Volção, L. M., & Junior, F. M. R. da S., 2020. Quais agrotóxicos estão contaminando os solos brasileiros? *Research, Society and Development*, 9(3), e114932569. <https://doi.org/10.33448/rsd-v9i3.2569>
- Gao, X., Yang, Y., & Wang, C. 2012. Geochemistry of organic carbon and nitrogen in surface sediments of coastal Bohai Bay inferred from their ratios and stable isotopic signatures. *Marine Pollution Bulletin*, 64(6), 1148–1155. <https://doi.org/10.1016/j.marpolbul.2012.03.028>
- Gatts, P.V., Franco, M.A.L., Almeida, M.G., Zalmon, I.R., Di Benedetto, A.P.M., Costa, P.A. S., Rezende, C.E., 2020. The trophic ecology of marine catfishes in south-eastern Brazil. *J. Mar. Biol. Assoc. U. K.* 100 (1), 133–142. <https://doi.org/10.1017/S0025315419001164>
- Gomes, P. R., Pestana, I. A., Viana, P. P., Almeida, M. G., Rezende, C. E., & Souza, C. M. M., 2022. Effects of dams on As and Hg concentrations in three southeastern Brazil fluvial systems: Ocean inputs, sources and seasonal dynamics among environmental compartments. *Science of the Total Environment*, 849. <https://doi.org/10.1016/j.scitotenv.2022.157865>
- Gomes, V. J. C., Freitas, P. T. A., & Asp, N. E., 2013. Dynamics and seasonality of the middle sector of a macrotidal estuary. *Journal of Coastal Research*, 165, 1140–1145. <https://doi.org/10.2112/si65-193.1>
- Gorayeb, A., Lombardo, M. A., & Pereira, L. C. C., 2009. Condições Ambientais em Áreas Urbanas da Bacia Hidrográfica do Rio Caeté – Amazônia Oriental - Brasil.

- Revista de Gestão Costeira Integrada*, 9(2), 59–70.
<https://doi.org/10.5894/rgci117>
- Gorayeb, A., Lombardo, M. A., & Pereira, L. C. C., 2011. Natural Conditions and Environmental Impacts in a Coastal Hydrographic Basin in the Brazilian Amazon. *Journal of Coastal Research*, 64, pg-pg.
www.jstor.org/stable/26482393
- Graves S, Piepho H, Dorai-Raj LSwhfS, 2024. _multcompView: Visualizations of Paired Comparisons_. R package version 0.1-10, <<https://CRAN.R-project.org/package=multcompView>>.
- Guimarães, D. O. (2011). *Condições Sócio-Ambientais da Bacia Hidrográfica do Rio Caeté (Pará, Brasil)* [Thesis]. Universidade Federal do Pará.
https://ppba.propesp.ufpa.br/ARQUIVOS/teses/2011/Tese_CondicoesSocioAmbientais.pdf
- Guo, Q., Wang, C., Wei, R., Zhu, G., Cui, M., & Okolic, C. P. (2020). Qualitative and quantitative analysis of source for organic carbon and nitrogen in sediments of rivers and lakes based on stable isotopes. *Ecotoxicology and Environmental Safety*, 195, 110436. <https://doi.org/10.1016/j.ecoenv.2020.110436>
- Gurgatz, B. M., Garcia, M. R., Cabral, A. C., de Souza, A. C., Nagai, R. H., Figueira, R. C. L., de Mahiques, M. M., & Martins, C. C., 2023. Polycyclic aromatic hydrocarbons in a Natural Heritage Estuary influenced by anthropogenic activities in the South Atlantic: Integrating multiple source apportionment approaches. *Marine Pollution Bulletin*, 188, 114678.
<https://doi.org/10.1016/j.marpolbul.2023.114678>
- Harrell, Jr. F. (2024). _Hmisc: Harrell Miscellaneous_. R package version 5.1-3, <<https://CRAN.R-project.org/package=Hmisc>>
- Hu, W., Lu, Y., Wang, T., Luo, W., Zhang, X., Geng, J., Wang, G., Shi, Y., Jiao, W., Chen, C., 2010. Factors affecting HCH and DDT in soils around watersheds of Beijing reservoirs, China. *Environ Geochem Health* 32, 85–94.
<https://doi.org/10.1007/s10653-009-9267-0>
- Kay, M., Elkin, L., Higgins, J., Wobbrock, J., 2025. _ARTool: Aligned Rank Transform for Nonparametric Factorial ANOVAs_. doi:10.5281/zenodo.594511 <<https://doi.org/10.5281/zenodo.594511>>, R package version 0.11.2

- Keil, R. G., & Mayer, L. M. (2014). Mineral Matrices and Organic Matter. In *Treatise on Geochemistry* (pp. 337–359). Elsevier. <https://doi.org/10.1016/b978-0-08-095975-7.01024-x>
- Lämmle, L., Perez Filho, A., Donadio, C., Arienzo, M., Ferrara, L., Santos, C. de J., & Souza, A. O., 2022 . Anthropogenic Pressure on Hydrographic Basin and Coastal Erosion in the Delta of Paraíba do Sul River, Southeast Brazil. *Journal of Marine Science and Engineering*, 10(11). <https://doi.org/10.3390/jmse10111585>
- Lapola, D. M., Martinelli, L. A., Peres, C. A., Ometto, J. P. H. B., Ferreira, M. E., Nobre, C. A., Aguiar, A. P. D., Bustamante, M. M. C., Cardoso, M. F., Costa, M. H., Joly, C. A., Leite, C. C., Moutinho, P., Sampaio, G., Strassburg, B. B. N., & Vieira, I. C. G., 2014. Pervasive transition of the Brazilian land-use system. *Nature Climate Change*, 4(1), 27–35. <https://doi.org/10.1038/nclimate2056>
- Li, D., Yan, J., Lu, Z., Chu, T., Li, J., & Chu, T., 2023. Use of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as Indicators to Evaluate the Influence of Sewage on Organic Matter in the Zhangjiang Mangrove–Estuary Ecosystem, Southeastern China. *Water*, 15(20), 3660. <https://doi.org/10.3390/w15203660>
- Li, G., Xia, X., Yang, Z., Wang, R., & Voulvoulis, N., 2006. Distribution and sources of polycyclic aromatic hydrocarbons in the middle and lower reaches of the Yellow River, China. *Environmental Pollution*, 144(3), 985–993. <https://doi.org/10.1016/j.envpol.2006.01.047>
- Maioli, O. L. G., Rodrigues, K. C., Knoppers, B. A., & Azevedo, D. A., 2011. Distribution and sources of aliphatic and polycyclic aromatic hydrocarbons in suspended particulate matter in water from two Brazilian estuarine systems. *Continental Shelf Research*, 31(10), 1116–1127. <https://doi.org/10.1016/j.csr.2011.04.004>
- Mao, S., Liu, S., Zhou, Y., An, Q., Zhou, X., Mao, Z., Wu, Y., & Liu, W., 2021. The occurrence and sources of polychlorinated biphenyls (PCBs) in agricultural soils across China with an emphasis on unintentionally produced PCBs. *Environmental Pollution*, 271, 116171. <https://doi.org/10.1016/j.envpol.2020.116171>
- Marques, J. S. J., Dittmar, T., Niggemann, J., Almeida, M. G., Gomez-Saez, G. V., & Rezende, C. E., 2017. Dissolved Black Carbon in the Headwaters-to-Ocean Continuum of Paraíba Do Sul River, Brazil. *Frontiers in Earth Science*, 5. <https://doi.org/10.3389/feart.2017.00011>

- Martinelli, L. A., Nardoto, G. B., Soltangheisi, A., Reis, C. R. G., Abdalla-Filho, A. L., Camargo, P. B., Domingues, T. F., Faria, D., Figueira, A. M., Gomes, T. F., Lins, S. R. M., Mardegan, S. F., Mariano, E., Miatto, R. C., Moraes, R., Moreira, M. Z., Oliveira, R. S., Ometto, J. P. H. B., Santos, F. L. S., ... Vieira, S. A., 2020. Determining ecosystem functioning in Brazilian biomes through foliar carbon and nitrogen concentrations and stable isotope ratios. *Biogeochemistry*, 154(2), 405–423. <https://doi.org/doi.org/10.1007/s10533-020-00714-2>
- Martins, C.C., Bicego, M.C., Rose, N.L., Taniguchi, S., Lourenço, R.A., Figueira, R.C.L., Mahiques, M.M., Montone, R.C., 2010. Historical record of polycyclic aromatic hydrocarbons (PAHs) and spheroidal carbonaceous particles (SCPs) in marine sediment cores from Admiralty Bay, King George Island, Antarctica. *Environmental Pollution* 158, 192–200. <https://doi.org/10.1016/j.envpol.2009.07.025>
- McLachlan, R. L., Ogston, A. S., Asp, N. E., Fricke, A. T., Nitttrouer, C. A., & Gomes, V. J. C., 2020. Impacts of tidal-channel connectivity on transport asymmetry and sediment exchange with mangrove forests. *Estuarine, Coastal and Shelf Science*, 233. <https://doi.org/10.1016/j.ecss.2019.106524>
- Mendiburu, F., 2023. *_agricolae: Statistical Procedures for Agricultural Research_*. R package version 1.3-7, <<https://CRAN.R-project.org/package=agricolae>>.
- Monteiro, M. C., Jiménez, J. A., & Pereira, L. C. C., 2016. Natural and human controls of water quality of an Amazon estuary (Caeté-PA, Brazil). *Ocean & Coastal Management*, 124, 42–52. <https://doi.org/10.1016/j.ocecoaman.2016.01.014>
- Nam, J. J., Gustafsson, O., Kurt-Karakus, P., Breivik, K., Steinnes, E., & Jones, K. C., 2008. Relationships between organic matter, black carbon and persistent organic pollutants in European background soils: Implications for sources and environmental fate. *Environmental Pollution*, 156(3), 809–817. <https://doi.org/10.1016/j.envpol.2008.05.027>
- Nascimento, R. A., de Almeida, M., Escobar, N. C. F., Ferreira, S. L. C., Mortatti, J., & Queiroz, A. F. S., 2017. Sources and distribution of polycyclic aromatic hydrocarbons (PAHs) and organic matter in surface sediments of an estuary under petroleum activity influence, Todos os Santos Bay, Brazil. *Marine Pollution Bulletin*, 119(2), 223–230. <https://doi.org/10.1016/j.marpolbul.2017.03.069>

- Ogle DH, Doll JC, Wheeler AP, Dinno A., 2023. *_FSA: Simple Fisheries Stock Assessment Methods_*. R package version 0.9.5, <<https://CRAN.R-project.org/package=FSA>>.
- Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J., 2024. *_vegan: Community Ecology Package_*. R package version 2.6-6.1, <https://CRAN.R-project.org/package=vegan>
- Oliveira, A. H. B., Cavalcante, R. M., Duaví, W. C., Fernandes, G. M., Nascimento, R. F., Queiroz, M. E. L. R., & Mendonça, K. V., 2016. The legacy of organochlorine pesticide usage in a tropical semi-arid region (Jaguaribe River, Ceará, Brazil): Implications of the influence of sediment parameters on occurrence, distribution and fate. *Science of The Total Environment*, 542, 254–263. <https://doi.org/10.1016/j.scitotenv.2015.10.058>
- Oliveira, D. M., Carvalho, V. S. B., da Silva, B. C., Reboita, M. S., & de Campos, B., 2023. Hydrological and Precipitation Extremes and Trends over the Paraíba do Sul River Basin, Brazil. *Climate*, 11(7), 138. <https://doi.org/10.3390/cli11070138>
- Ometto, J. P. H. B., Ehleringer, J. R., Domingues, T. F., Berry, J. A., Ishida, F. Y., Mazzi, E., Higuchi, N., Flanagan, L. B., Nardoto, G. B., & Martinelli, L. A., 2006. The stable carbon and nitrogen isotopic composition of vegetation in tropical forests of the Amazon Basin, Brazil. *Biogeochemistry*, 79(1–2), 251–274. <https://doi.org/doi.org/10.1007/s10533-006-9008-8>
- Park, H. J., Baek, N., Lim, S. S., Jeong, Y. J., Seo, B. S., Kwak, J. H., Lee, S. M., Yun, S. I., Kim, H. Y., Arshad, M. A., & Choi, W. J., 2023. Coupling of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to understand soil organic matter sources and C and N cycling under different land-uses and management: a review and data analysis. In *Biology and Fertility of Soils* (Vol. 59, Issue 5, pp. 487–499). Springer Science and Business Media Deutschland GmbH. <https://doi.org/10.1007/s00374-022-01668-3>
- Parween, M., Ramanathan, AL., Khillare, P. S., & Raju, N. J., 2014. Persistence, variance and toxic levels of organochlorine pesticides in fluvial sediments and the role of black carbon in their retention. *Environmental Science and Pollution Research*, 21(10), 6525–6546. <https://doi.org/10.1007/s11356-014-2531-6>

- Pereira, L. C. C., Sousa, N. S. S., Rodrigues, L. M. S., Monteiro, M. C., Silva, S. R. S., Oliveira, A. R. G., Dias, A. B. B., & Costa, R. M., 2021. Effects of the lack of basic public sanitation on the water quality of the Caeté River estuary in northern Brazil. *Ecohydrology and Hydrobiology*, 21(2), 299–314. <https://doi.org/10.1016/j.ecohyd.2020.12.003>
- Pfeiffer, W. C., Fiszman, M., Malm, O., & Azcue, J. M., 1986. Heavy metal pollution in the Paraíba do sul river. *The Science of the Total Environment*, 58, 73–79. [https://doi.org/doi.org/10.1016/0048-9697\(86\)90077-X](https://doi.org/doi.org/10.1016/0048-9697(86)90077-X)
- Pinto, L. F. G., Ferreira, J., Berenguer, E., & Rosa, M., 2023. Governance lessons from the Atlantic Forest to the conservation of the Amazon. *Perspectives in Ecology and Conservation*, 21(1), 1–5. <https://doi.org/10.1016/J.PECON.2022.10.004>
- Prado, M. R. do, Carvalho, D. R. de, Alves, C. B. M., Moreira, M. Z., & Pompeu, P. S., 2020. Convergent responses of fish belonging to different feeding guilds to sewage pollution. *Neotropical Ichthyology*, 18(1). <https://doi.org/10.1590/1982-0224-2019-0045>
- R Core Team., 2024. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.R-project.org>
- Raiol, L. L., Santos, D. C. R., Santos, D. S. dos, Silva, A. D. C. da, & Júnior, J. F. da S., 2019. Uso e ocupação do solo e áreas de preservação permanente na Bacia Hidrográfica do Rio Caeté, Pará. *Meio Ambiente em Foco - Volume 10*. Editora Poisson. <https://doi.org/10.36229/978-85-7042-189-0.cap.13>
- Ribas, L. M., 2012. *Caracterização de fontes de matéria orgânica do estuário do rio Paraíba do Sul, RJ, Brasil* [Thesis, Universidade Estadual do Norte Fluminense]. <https://uenf.br/posgraduacao/ecologia-recursosnaturais/wp-content/uploads/sites/7/2013/11/Ligia-Macabu-Ribas-vers%C3%A3o-final.pdf>
- Santos, F.R., Neves, P.A., Kim, B.S.M., Taniguchi, S., Lourenço, R.A., Timoszczuk, C.T., Bicego, M.C., 2020. Organic contaminants and trace metals in the western South Atlantic upper continental margin: anthropogenic influence on mud depocenters. *Mar. Pollut. Bull.* 154, 111087. <https://doi.org/10.1016/j.marpolbul.2020.111087>.
- Santos-Andrade, M., Hatje, V., Arias-Ortiz, A., Patire, V. F., & da Silva, L. A., 2021. Human disturbance drives loss of soil organic matter and changes its stability and sources in mangroves. *Environmental Research*, 202. <https://doi.org/10.1016/j.envres.2021.111663>

- Sartori, É., Vergilio, C. S., Gatts, P. V., Oliveira, B. C. V., Almeida, M. G., Thompson, F., & Rezende, C. E., 2023. Trace metal concentration along the Brazilian coast: An assessment of the influence of the Doce River plume. *Marine Pollution Bulletin*, 188. <https://doi.org/10.1016/j.marpolbul.2023.114640>
- Sebastien Le, Julie Josse, Francois Husson, 2008. FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*, 25(1), 1-18. [10.18637/jss.v025.i01](https://doi.org/10.18637/jss.v025.i01)
- Serafim, T. S. G., Almeida, M. G., Thouzeau, G., Michaud, E., Niggemann, J., Dittmar, T., Seidel, M., & Rezende, C. E., 2023. Land-use changes in Amazon and Atlantic rainforests modify organic matter and black carbon compositions transported from land to the coastal ocean. *Science of The Total Environment*, 878, 162917. <https://doi.org/10.1016/j.scitotenv.2023.162917>
- Shi, L., Ortals, C., Valle-Levinson, A., & Olabarrieta, M., 2023. Influence of river discharge on tidal and subtidal flows in a microtidal estuary: Implication on velocity asymmetries. *Advances in Water Resources*, 177, 104446. <https://doi.org/10.1016/j.advwatres.2023.104446>
- Solórzano, A., Brasil, L. S. C. A., & Oliveira, R. R., 2021. The Atlantic Forest Ecological History: From Pre-colonial Times to the Anthropocene. In *The Atlantic Forest: History, Biodiversity, Threats and Opportunities of the Mega-diverse Forest* (pp. 25–44). Springer, Cham. https://doi.org/10.1007/978-3-030-55322-7_2
- Souza, A. C., Combi, T., da Silva, J., & Martins, C. C., 2018a. Occurrence of halogenated organic contaminants in estuarine sediments from a biosphere reserve in Southern Atlantic. *Marine Pollution Bulletin*, 133, 436–441. <https://doi.org/10.1016/j.marpolbul.2018.05.052>
- Souza, A. C., Taniguchi, S., Lopes Figueira, R. C., Montone, R. C., Caruso Bícago, M., & Martins, C. C., 2018b. Historical records and spatial distribution of high hazard PCBs levels in sediments around a large South American industrial coastal area (Santos Estuary, Brazil). *Journal of Hazardous Materials*, 360, 428–435. <https://doi.org/10.1016/j.jhazmat.2018.08.041>
- Sreedevi, M. A. and Harikumar, P. S., 2023. Occurrence, distribution, and ecological risk of heavy metals and persistent organic pollutants (OCPs, PCBs, and PAHs) in surface sediments of the Ashtamudi wetland, south-west coast of India. *Regional Studies in Marine Science*, 64, 103044. <https://doi.org/10.1016/j.rsma.2023.103044>

- Stock BC, Jackson AL, Ward EJ, Parnell AC, Phillips DL, Semmens BX. 2018. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. *PeerJ* 6:e5096 <https://doi.org/10.7717/peerj.5096>
- Tang, D., Liu, X., He, H., Cui, Z., Gan, H., Xia, Z., 2020. Distribution, sources and ecological risks of organochlorine compounds (DDTs, HCHs and PCBs) in surface sediments from the Pearl River Estuary, China. *Marine Pollution Bulletin* 152, 110942. <https://doi.org/10.1016/j.marpolbul.2020.110942>
- Thornton, S. F., and McManus, J., 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: Evidence from the Tay Estuary, Scotland. *Estuarine, Coastal and Shelf Science*, 38, 219-233.
- Tonh , M. S., Ara jo, D. F., Ara jo, R., Cunha, B. C. A., Machado, W., Portela, J. F., PR Souza, J., Carvalho, H. K., Dantas, E. L., Roig, H. L., Seyler, P., & Garnier, J., 2021. Trace metal dynamics in an industrialized Brazilian river: A combined application of Zn isotopes, geochemical partitioning, and multivariate statistics. *Journal of Environmental Sciences*, 101, 313–325. <https://doi.org/10.1016/j.jes.2020.08.027>
- Turubanova, S., Potapov, P. V., Tyukavina, A., & Hansen, M. C., 2018. Ongoing primary forest loss in Brazil, Democratic Republic of the Congo, and Indonesia. *Environmental Research Letters*, 13(7), 074028. <https://doi.org/10.1088/1748-9326/AACD1C>
- Venables, W.N. and Ripley, B.D., 2002. Modern Applied Statistics With S, Fourth edition. Springer, New York, ISBN 0-387-95457-0
- Vezzone, M., Meigikos dos Anjos, R., Gon alves Cesar, R., Muniz, M., Cardoso, R., Paulo Felizardo, J., Vasconcelos, D., Polivanov, H., & Vezzone mvezzzone, M., 2021. Using stable isotopes to discriminate anthropogenic impacts of the sedimentary organic matter pollution in the Rodrigo de Freitas Lagoon (RJ, Brazil). *Environmental Science and Pollution Research*. <https://doi.org/doi.org/10.1007/s11356-020-10835-8>
- Ward, N.D., Bianchi, T.S., Medeiros, P.M., Seidel, M., Richey, J.E., Keil, R.G., Sawakuchi, H.O., 2017. Where Carbon Goes When Water Flows: Carbon Cycling across the Aquatic Continuum. *Front. Mar. Sci.* 4. <https://doi.org/10.3389/fmars.2017.00007>

- Weirich, Y. S. V., Rezende, C. E., Souza, F. M., Santos, V. F., Martins, C. C., 2025. Unraveling sedimentary organic matter in the Oiapoque estuarine-marine ecosystem: Insights from elemental composition and isotopic fingerprints on the Amazon coast. *Marine Chemistry* 273, 104565. <https://doi.org/10.1016/j.marchem.2025.104565>
- Yang, H., Xue, B., Yu, P., Zhou, S., & Liu, W., 2010. Residues and enantiomeric profiling of organochlorine pesticides in sediments from Yueqing Bay and Sanmen Bay, East China Sea. *Chemosphere*, 80(6), 652–659. <https://doi.org/10.1016/j.chemosphere.2010.04.052>
- Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Organic Geochemistry* 33, 489–515. [https://doi.org/10.1016/s0146-6380\(02\)00002-5](https://doi.org/10.1016/s0146-6380(02)00002-5)
- Zhao, J., Zhang, L., Zhang, Y., Yu, Q., & Luo, S. (2024). Lipid biomarker evidences of natural and anthropogenic organic matter inputs in sediments from the eastern Sunda Shelf in the southern South China Sea. *Continental Shelf Research*, 275, 105184. <https://doi.org/10.1016/j.csr.2024.105184>

Supplementary Material

Link: <https://ars.els-cdn.com/content/image/1-s2.0-S0025326X26006867-mmc1.docx>

CAPÍTULO 2:

Identifying drivers of sediment toxicity to a benthic copepod in tropical estuaries through contaminant mixture modeling

Submetido - **Journal of Hazardous Materials**

Qualis-Capes (Área de Biodiversidade, 2021-2024): A1

Fator de Impacto (2025): 10.6

Status: Em revisão

CAPÍTULO 2 - Drivers of sediment toxicity in tropical estuaries through contaminant mixture modeling

Échily Sartori^a, Cristiane dos Santos Vergilio^b, Jéssica Dutra Ferreira^b, Ingrid Ferreira dos Santos^b, Ana Luiza Gonçalves Sepulcro^b, Diego Lacerda^a, Luiza Silva do Nascimento^a, Pedro Vianna Gatts^a, Jamile Tamer Nasser^c, César de Castro Martins^c, Erika Kurahashi^d, Lukas Klose^d, Andrea Koschinsky^d, Nils E. Asp^e, Carlos Eduardo de Rezende^a

^a Grupo de Pesquisa em Biogeoquímica de Ecossistemas Aquáticos, Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro. Avenida Alberto Lamego, 2000, Parque Califórnia, Campos dos Goytacazes, Rio de Janeiro, 28013-602, Brazil.

^b Laboratório de Ecotoxicologia, Departamento de Biologia, Centro de Ciências Exatas Naturais e da Saúde, Universidade Federal do Espírito Santo - Campus Alegre. Alto Universitário, S/N, Guararema, Alegre, Espírito Santo, 29.500-000, Brazil.

^c Laboratório de Química Orgânica Marinha, Instituto Oceanográfico, Universidade de São Paulo (USP), Praça do Oceanográfico, 191, Cidade Universitária, São Paulo, 05508-120, Brazil

^d OceanLab, Department of Physics and Earth Science, School of Science, Constructor University, Bremen, Germany

^e Instituto de Estudos Costeiros (IECOS), Universidade Federal do Pará (UFPA), Campus de Bragança, Pará, Brazil

Abstract

Tropical estuaries and mangroves concentrate contaminants from multiple anthropogenic sources. Nevertheless, identifying compounds and complex mixtures drive toxicity, remains a central ecotoxicological challenge. This study investigated contaminant mixture effects on the reproductive output of the benthic copepod *Nitokra* sp. in sediments from two contrasting Brazilian tropical basins: the Caeté River (CR), under diffuse anthropogenic pressures, and the Paraíba do Sul River (PSR), impacted by decades of industrial and agricultural activities. Surface sediments were characterized by trace metals, PAHs, PCBs, and OCPs, and 10-day chronic bioassays were conducted. Contaminant concentrations were consistently higher in PSR basin, with mangrove soils acting as preferential sinks in both basins. Offspring production decreased significantly in PSR mangrove soils (up to 7-fold lower than control),

followed by CR mangrove (~2.4-fold lower than control). When contaminant classes were evaluated as simultaneous one-quartile shifts across all compounds, PCBs and OCPs emerged as the strongest drivers of reproductive impairment (–43.3% and –39.3%, respectively), followed by PAHs, while no individual group of contaminants was statistically significant in isolation. The metals group was positively associated with reproduction, primarily reflecting the protective effects of calcium, which outweighed the toxic effects of the other metals. Most identified individual contaminant drivers, including PCB138, p,p'-DDE, and chrysene, occurred below established sediment quality guidelines, demonstrating that mixture-level toxicity emerges well below individual regulatory thresholds. These findings reveal that ecotoxicologically relevant contamination goes undetected by single-compound regulatory frameworks, highlighting the need for mixture-based sediment risk assessment.

Key-words: Caeté River, OCP, PAH, Paraíba do Sul River, PCB

Introduction

Tropical estuaries associated with mangrove forests are among the most productive and ecologically relevant ecosystems on earth, due to the large amounts of organic matter (OM) produced in mangroves and exported to estuarine and coastal regions, which sustains biodiversity and food web productivity (Bernini and Rezende, 2011; Bayen, 2012; Silva et al., 2023). These environments function as breeding, nursery, and feeding grounds for numerous species of invertebrates and fish, in addition to providing a wide range of ecosystem services (Mbusnum et al., 2020; Lugendo and Kimirei, 2021). However, because they occupy the transitional zone between the continent and the ocean, within sediment transport convergence and strong sedimentation trends, estuaries and mangroves receive contaminant inputs from drainage basins via surface runoff, which tend to accumulate in bottom sediments, threatening the ecological functions of these systems (Bayen, 2012; Lugendo and Kimirei, 2021; Silva et al., 2023).

Rapid population growth, industrialization, and port expansion in these areas place these systems among the most vulnerable, with increasing discharges of effluents rich in potentially toxic elements and compounds (PTEC) such as trace metals, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), and polychlorinated biphenyls (PCBs) to both ecosystem and human health (Tang et

al., 2020; Çelebi et al., 2024). These contaminants can persist in sediments for years due to their resistance to biological and chemical degradation or be remobilized into the water column following changes in the physicochemical composition of sediments (Barhoumi et al., 2019; Çelebi et al., 2024). Metals such as Hg, As, and Pb deserve particular attention due to their bioaccumulation and biomagnification potential (Silva et al., 2023), and especially PAHs and persistent organic pollutants (POPs, represented by DDT-class OCPs and PCBs), due to their carcinogenic, mutagenic, and teratogenic potential, in addition to being globally banned or severely restricted under the Stockholm Convention (UNEP, 2001; Bayen, 2012; Çelebi et al., 2024), and nationally prohibited in terms of production, use, and commercialization of PCBs (Brazil, 1981) and DDTs (Brazil, 1985, 2009).

In this context, ecotoxicology is essential for assessing the impacts of these environmental issues by measuring the potential biological effects caused by complex contaminant mixtures present in sediments, since only the determination of contaminant concentrations in sediment is insufficient to predict their effects on biota (Vergilio et al., 2020 and 2021; Gu et al., 2022; Silva et al., 2023). Laboratory assays using copepods benthic organisms, such as those of the genus *Nitokra*, are ideal bioindicators because they are typical of tropical coastal regions, live in direct contact with sediments, serve as an energy base for higher trophic levels, have short life cycles, are easy to culture, and are highly sensitive to a wide range of stressors (Lotufo and Abessa, 2002; Ward et al., 2011; Silva et al., 2023; Monte et al., 2025).

In Brazil, two tropical estuarine systems stand out due to the contrast in their contamination histories (Sartori et al., 2026). The Caeté River (CR) basin, located in northern Brazil and part of the Amazonian hydrographic system, and the Paraíba do Sul River (PSR) basin, located in the southeastern region of the country, one of the most industrialized and densely populated areas in Brazil. While the CR faces environmental pressures mainly associated with inadequate sanitation, agricultural activities, and fisheries activities (Gorayeb et al., 2009, 2011; Sartori et al., 2026), the PSR carries decades of industrial and domestic effluent discharge, intensive agrochemical use, and environmental accidents involving industrial spills, resulting in a documented legacy of sediment contamination (Pfeiffer et al., 1986; AGEVAP, 2020; Sartori et al., 2026). Contaminant loads in these systems are well documented by Sartori et al. (2026), who detected PAH, PCB, and OCP (DDT-class) concentrations in both estuarine systems.

Despite the number of studies reporting contamination by metals, PAHs, PCBs, and OCPs in tropical estuarine sediments (e.g., Oliveira et al., 2014; 2016; Souza et al., 2018a, b; Silva et al., 2023; Sartori et al., 2023), it remains poorly understood which contaminant classes are the main drivers of toxic effects on biota, especially when these compounds co-occur in complex mixtures. Historically, sediment risk assessment has evolved through distinct paradigms: early approaches relied solely on measuring contaminant concentrations against regulatory guidelines, then ecotoxicological bioassays were introduced to capture direct biological responses but were typically interpreted apart from chemical data (Sisinno and Oliveira-Filho, 2013; Vergilio et al., 2020 and 2021).

Despite this advance, assessing the toxic effects of contaminant mixtures represents one of the greatest challenges in contemporary environmental ecotoxicology. Traditional approaches based on bivariate correlations or univariate analyses are insufficient to capture the joint effects of multiple co-occurring compounds, particularly under conditions of high collinearity. Recently, methods developed in environmental epidemiology, such as quantile g-computation (*qgcomp*), have been applied to estimate the combined effect of exposure mixtures, providing unbiased estimates even under limited sample sizes and allowing mixture components to act in opposing directions (Keil et al., 2020; Yu et al., 2022). The integration of *qgcomp* with Elastic Net (EN) regression and principal component analysis (PCA) represents a significant methodological advance, enabling the handling of high dimensionality and multicollinearity, and strengthening an innovative approach still underexplored in sediment ecotoxicology.

Accordingly, the present study sought to answer the following questions: (i) how do the concentrations of metallic and organic contaminants in estuarine and mangrove sediments differ between two Brazilian tropical basins with contrasting contamination histories? (ii) Is the reproductive output of benthic copepods reduced in sediments from more contaminated environments? and (iii) which contaminant classes and individual compounds are most strongly associated with reproductive impairment when evaluated as complex mixtures? To address these questions, sediment samples from the Caeté River and Paraíba do Sul River basins were subjected to chemical characterization and ecotoxicological assays with *Nitokra* sp., followed by an integrative analytical framework combining generalized linear mixed models, quantile g-computation, Elastic Net regression, and principal component analysis. By

integrating these complementary approaches, this study moves beyond the traditional identification of contaminated sites toward identifying the contaminant classes and individual compounds strongly associated with biological impairment. This framework improves the interpretation of sediment toxicity by disentangling the relative contribution of co-occurring contaminants and provides a transferable analytical strategy for prioritizing contaminants in complex environmental mixtures, thereby supporting more effective ecological risk assessment and sediment management.

Material and Methods

Study Area and Sampling

Twenty-four estuarine surface sediment and thirty mangrove soil samples were collected during dry and rainy seasons in two contrasting tropical basins: the CR basin, in northern Brazil, and the PSR basin, in southeastern Brazil, between 2022 and 2023, totaling 54 samples (Fig. 1). The sampling design was structured to compare contaminant mixtures and sediment-associated toxicity between basins, environments, and seasons. Samples were collected, preserved, and prepared following the procedures described in Sartori et al. (2026), which provides additional details on field protocols and site characterization.

The CR basin covers approximately 2,230 km² in northeastern Pará State and is part of the broader Amazon hydrographic system. Rainfall follows a marked seasonal pattern, with a wet period extending from December to August and a pronounced dry season from September to November. The system is macro-tidal, with spring tides of about 5 m (Dittmar et al., 2001; Abdala et al., 2012; Asp et al., 2018). The PSR basin, in contrast, drains one of the most urbanized and industrialized corridors in Brazil, covering approximately 56,900 km². Its climate is also strongly seasonal, alternating between a dry period (April to October) and a rainy season (late October to March).

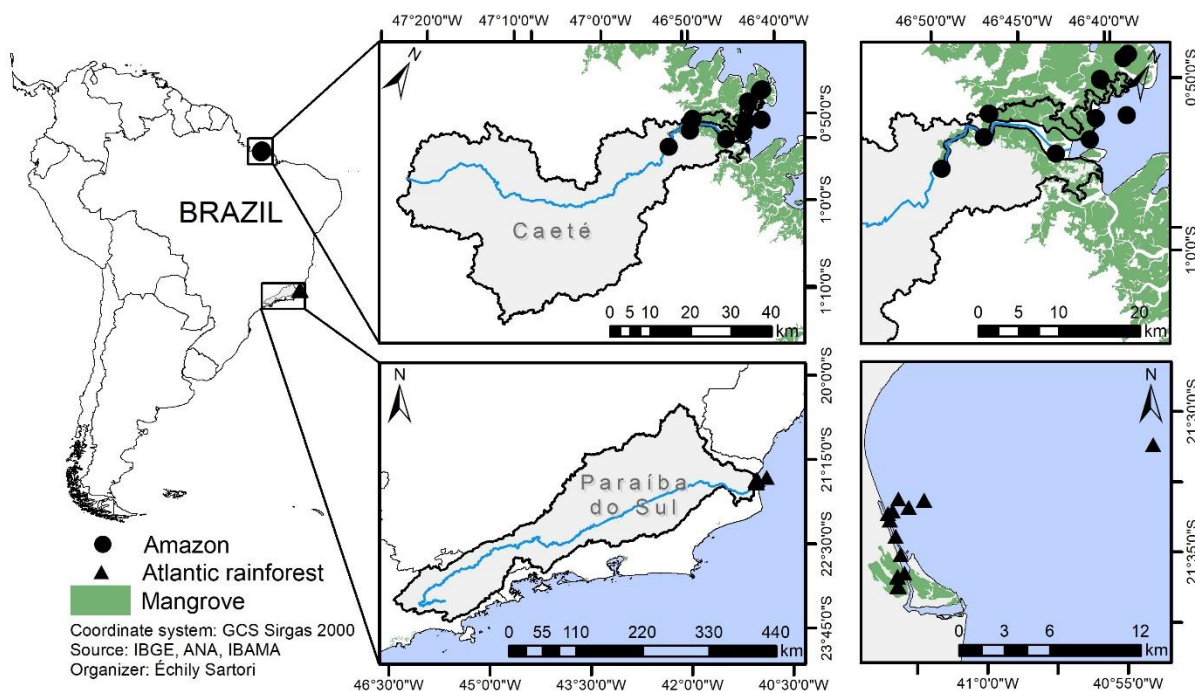


Fig.: 1 Design of sediments samples across the Caeté River (CR) and Paraíba do Sul River (PSR) basin. Reproduced from Sartori et al. (2026).

Sediment Characterization

Samples were freeze-dried and sieved to <2.0 mm grain size determination by laser diffraction (Shimadzu SALD-3101), and the median grain size (MGS) was obtained using the Folk and Ward (1957) graphical method via the SYSGRAN software (Camargo, 2006). The procedures for organic carbon (OC) content were described in Sartori et al. (2026), where 10 mg of decarbonated, sediments were weighed in silver capsules and analyzed using an elemental analyzer (Flash 2000) coupled to an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific) via a CONFLO IV interface, with acetanilide as the quantification standard. OC content was expressed as a percentage (detection limit: 0.05%).

Total Metal Determination

To remove residual moisture, approximately 0.05 g of freeze-dried sediment samples were oven-dried at 105°C overnight. Subsequently, 3 mL of HF and 3 mL of HClO₄ were added, and the samples were digested in a pressurized block digester (Picotrace Block®) at 235°C for approximately 18h. After digestion, the solutions were cooled (<60°C) and the system depressurized. The acids then nearly completely

evaporated under the same temperature conditions, avoiding total dryness of the samples. Next, 5 mL of HCl 30% (technical grade) was added, and the solution was evaporated, repeating this step once. Finally, the samples were reconstituted in 10 mL of 0.5 mol L⁻¹ HNO₃, left to rest for a few minutes, and transferred to 50 mL flasks and made up to volume.

Measurement of metals (Cd, Cr, Cu, Fe, Ni, Pb and Zn) and As concentration were performed by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS, PerkinElmer, NexION 350X). Measurement of macronutrient (Ca) was performed by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES – Spectro, Ciros Vision) at Constructor University, Germany. Instrumental quantification limits (QLs) were defined as ten times the standard deviation of procedural acid blanks (0.035 µg·kg⁻¹ for As, 145 mg·kg⁻¹ for Ca, 0.070 mg·kg⁻¹ for Cd, 0.025 µg·kg⁻¹ for Cr, 0.066 µg·kg⁻¹ for Cu, 0.477 µg·kg⁻¹ for Fe, 0.255 mg·kg⁻¹ for Ni, 0.015 mg·kg⁻¹ for Pb and 0.031 µg·kg⁻¹ for Zn).

POPs Determination

PAHs (naphthalene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, benzo[g,h,i]perylene, C₂-naphthalene, C₃-naphthalene, C₁-dibenzothiophene, C₁-phenanthrene, C₂-phenanthrene and C₁-pyrene), PCBs (PCB18, 44, 52, 66, 101, 138), and OCPs (γ-HCH, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT) were extracted from 15 g of freeze-dried sediment and quantified following Santos et al. (2020). Identification and quantification were performed by GC-MS/MS in MRM mode using certified calibration standards. QLs corresponded to the lowest calibration levels: (0.006 ng·g⁻¹ for PAHs, 0.001 ng·g⁻¹ for PCBs and 0.002 ng·g⁻¹ for OCPs). Instrumental methods and quality assurance details were described by Sartori et al. (2026).

Quality Control and Assurance

For metal and As analysis labware was acid-cleaned prior to use. Two analytical blanks and reference materials (NIST 1645, NIST 2702 and SRLS-6) from National Bureau of Standards, National Institute of Standards and Technology and National Research Council Canada were also used for quality control and to ensure accuracy

of measurements. Recovery values for the standards varied between 90 and 110% for all elements.

For POPs, procedures included blanks (no interferences detected), surrogate recoveries (50–110%), spiked blanks and samples recoveries (50–110%), and certified reference material (IAEA-417) from International Atomic Energy Agency, with measured concentrations within 60–90% (PAHs) and 75–90% (PCBs/OCPs) of certified values.

Toxicity Test

Ecotoxicological tests using the copepods *Nitokra* sp., cultivated at the Laboratory of Ecotoxicology from Federal University of Espírito Santo (UFES), were carried out according to adapted method of Lotufo and Abessa (2002). The tests were conducted with 10 replicates per sampling site, using approximately 2 g of sample corresponding to about 0.5 mm in height (sediment or soil), and completed with 15 mL of reconstituted seawater (Red Sea[®]) with salinity 35. A fixed salinity (33 ± 2) was used in all assays to standardize experimental conditions and minimize osmotic stress on the test organism. Five ovigerous females were added to each tube. Although this standardized salinity does not fully reflect the natural spatial and temporal salinity variability observed among sampling sites, it was adopted to ensure consistent test conditions and reduce the influence of salinity-related physiological stress on toxicity responses. For control treatment, a sediment composed of coarse sand, previously sieved through a 2 mm mesh, washed in reconstituted seawater (salinity 35) and dried at room temperature, was used. The samples were left to stand 24 hours before the start of the tests. Following the addition of the females, each tube was fed once with 0.1 mL of a suspension containing algae suspension (10^5 cells mL⁻¹) and incubated at 24 ± 2 °C for ten days in a BOD chamber (SP LABOR/SP-224) under a 16:8 h light:dark photoperiod.

Physical-chemical parameters (*i.e.*, dissolved oxygen [DO], pH, temperature and salinity) were measured at the beginning and at the end of the experiment to ensure that test conditions remained stable throughout the exposure period and did not affect organism responses. After 10 days, 0.5 mL of a solution of 10% formaldehyde and rose bengal dye (4%) was added to each tube for 48 h to fix and stain. Samples

were then sieved through a 45 μm mesh. Ovigerous, adult, and offspring (nauplii and copepodites) were counted.

Statistical Analysis

Statistical analyses were designed to evaluate spatial and seasonal patterns in contaminant concentrations and to relate these chemical patterns to reproductive responses in *Nitokra* sp. Prior to statistical modeling, values below the QLs were replaced by the corresponding QL value. Multiple QLs were available only for metal variables, with only Ca presenting one value below QL, which the highest value was applied. For all organic compound classes, a single QL was defined by analyte. Thus, the potential bias introduced is considered negligible. To ensure analytical representativeness, only variables with quantifiable detections in at least 70% of samples were retained for further analysis.

To compare contaminant concentrations between basins, one-way ANOVA was applied (aov, base package, R Core Team, 2024). Within each basin, the combined effects of sampling environment (estuary and mangrove), seasonality, and their interaction were assessed using two-way ANOVA. When residuals violated normality assumptions, a non-parametric two-way approach was employed through the Aligned Rank Transform method (art, ARTool package, Kay et al., 2025). Post-hoc comparisons were selected according to the model fitted for each variable: Tukey HSD (HSD.test, agricolae package, Mendiburu, 2023) was used after parametric ANOVA, whereas Dunn's test with Bonferroni correction was applied after rank-based analyses (dunnTest, FSA package, Ogle et al., 2023). Compact letter displays were generated using the multcompView package (multcompLetters, Graves et al., 2024).

Models' assumptions were validated using diagnostic plots (Altman and Krzywinski, 2016), and when necessary, data were transformed to meet ANOVA premises (linearity, normality, homoscedasticity, leverage), using a maximum likelihood function (boxcox, MASS package, Venables and Ripley, 2002). All analyses were conducted at a 95% confidence level. All statistics and analyses used in this study were performed using R version 4.4.0 (R Core Team, 2024).

Generalized Mixed Effect Models

Reproductive outputs were analyzed using Generalized Linear Mixed Models (GLMMs; glmmTMB package; Brooks et al., 2017). The primary response variable was offspring production, calculated as the total number of nauplii and copepodites produced per replicate after 10 days of exposure. Candidate distributions were compared using the Akaike Information Criterion (AIC), and model adequacy was assessed based on AIC and simulation-based residual diagnostics using the DHARMA package (Hartig, 2024), including tests for uniformity, dispersion, outliers, and zero inflation. The fixed-effect structure included Basin, Season, Environment (estuary and mangrove), and their interactions. To account for pseudoreplication arising from multiple replicates per sampling site, a random intercept was specified for each unique Basin × Season × Environment × Site combination. A second random intercept for Experiment was included to account for inter-assay variability among experimental batches. Model significance was assessed using Type III ANOVA (Anova, car package; Fox, 2019), and post-hoc pairwise comparisons were performed using estimated marginal means (emmeans package; Lenth, 2024) with Tukey adjustment at a 95% confidence level.

Potential Toxicity of Mixtures

To integrate chemical contamination with biological responses, contaminant concentrations and reproductive outputs were summarized at the site level. Offspring production was normalized relative to experimental controls to reduce the influence of inter-assay variability. Because sediment characteristics can influence both contaminant accumulation and biological responses, all models were adjusted for key sediment and contextual variables, including Fe, OC content, median grain size, and the Basin × Environment interaction.

The mixture analysis was designed to answer three complementary questions: (i) whether each contaminant class was associated with reduced reproductive output; (ii) which individual compounds within each class contributed most consistently to this association; and (iii) which contaminant classes were most relevant when all classes were evaluated together. Contaminants were grouped into six classes: metals, low-molecular-weight (LMW-PAHs), high-molecular-weight (HMW-PAHs) and alkylated PAHs, PCBs, and organochlorine pesticides (OCPs).

First, quantile g-computation (qgcomp.noboot, qgcomp package, Keil, 2025) was used to estimate the joint association between each contaminant class and offspring production. This approach evaluates how reproduction changes when all compounds within a class increase simultaneously by one quartile, rather than interpreting each compound in isolation. The mixture effect parameter (Ψ) represents the expected change in log-transformed offspring production per simultaneous one-quartile increase across all contaminants within a class, adjusted for confounders, and was back-transformed as $[\exp(\Psi) - 1] \times 100$ to express the estimated percent change in reproductive output. Individual component weights (w_j) were interpreted as directional indicators of each contaminant's contribution to the class-level mixture effect. To evaluate whether the positive association of Ca influenced the metals and As mixture estimate, the metals model was re-run excluding Ca. Second, Elastic Net regression ($\alpha = 0.5$; glmnet package, Friedman et al., 2010) was used to identify compounds that were consistently selected as potential drivers within each class, while reducing instability caused by collinearity among contaminants. Third, PCA (prcomp, base package, R Core Team, 2024) was used to identify compounds that contributed most strongly to the main concentration gradients within each class.

Evidence from these three approaches was then combined in a triangulation framework. Compounds supported by all methods were classified as robust drivers, whereas compounds supported by fewer lines of evidence were classified as probable drivers or candidates. Because of the limited sample size and the strong collinearity among contaminants, individual weights and coefficients were interpreted as directional indicators of association, not as precise causal effect estimates. Full model specifications, diagnostic procedures, and selection criteria are provided in the Supplementary Material.

Results

Sediment Contamination Across Tropical Estuarine Systems

Given the large number of variables analyzed, full concentration data and statistical comparisons are provided in the Supplementary Material (Fig. S1 to S6). For clarity, the following section presents only the compounds that were subsequently identified as potential toxicity drivers through the mixture modeling framework

described in Section 3.3, allowing the examine their spatial and temporal distribution patterns before the mixture analysis results are presented.

Briefly, overall contaminant concentrations were consistently and significantly higher in the PSR basin across nearly all classes ($p < 0.05$; Fig. S1 to S6). Within the CR basin, seasonal differences were generally absent, although environmental differences were detected for several classes, with higher concentrations in mangrove than estuarine sediments. For specific contaminants (phenanthrene, anthracene, fluoranthene, pyrene, benzo[*b*]fluoranthene, benzo[*e*]pyrene, C₁-dibenzothiophene and C₂-phenanthrene), a significant environment $\times$ season interaction was observed, with peak concentrations consistently associated with mangrove soils during both seasons, followed by estuarine sites. In the PSR basin, differences across seasons, environments, and their interaction were largely absent, and when detected, higher concentrations were again found in mangrove soils.

Among these, Pb, Naphthalene, Chrysene, C₁-Phenanthrene, PCB138, and *p,p'*-DDE showed distinct concentration patterns across basins, environments, and seasons (Fig. 2). At the basin level, PSR presented significantly higher concentrations than CR across all selected compounds ($p < 0.05$), with mean values approximately 1.6-fold higher for Pb, 5.7-fold for Naphthalene, 2.0-fold for C₁-Phenanthrene, 1.8-fold for *p,p'*-DDE, 9.4-fold for PCB138, and ~34-fold for Chrysene (Fig. 2a, d, g, j, m, p).

Within the CR basin, Pb, Naphthalene, Chrysene, and C₁-Phenanthrene shared a consistent pattern, with no significant seasonal differences but markedly higher concentrations in mangrove than estuarine sediments, with mean values approximately 3.9-, 3.2-, 3.1-, and 2.3-fold higher, respectively (Fig. 2b, e, h, k). PCB138 showed a distinct pattern, with the highest mean concentrations recorded in estuarine samples during the rainy season (0.024 ng·g⁻¹), followed by mangrove during the dry season (0.016 ng·g⁻¹), mangrove during the rainy season (0.012 ng·g⁻¹) and estuarine sediments during the dry season (0.006 ng·g⁻¹; Fig. 2n). For *p,p'*-DDE, mangrove presented mean concentrations approximately 5.1-fold higher than estuarine samples, with dry season values 1.4-fold higher than those recorded during the rainy season (Fig. 2q). In the PSR basin, no significant differences were detected across seasons or environments for any of the selected compounds (Fig. 2c, f, i, l, o), with the exception of *p,p'*-DDE (Fig. 2r), which showed higher concentrations in

mangrove sediments, with mean values approximately 2.3-fold above those recorded in estuarine sites.

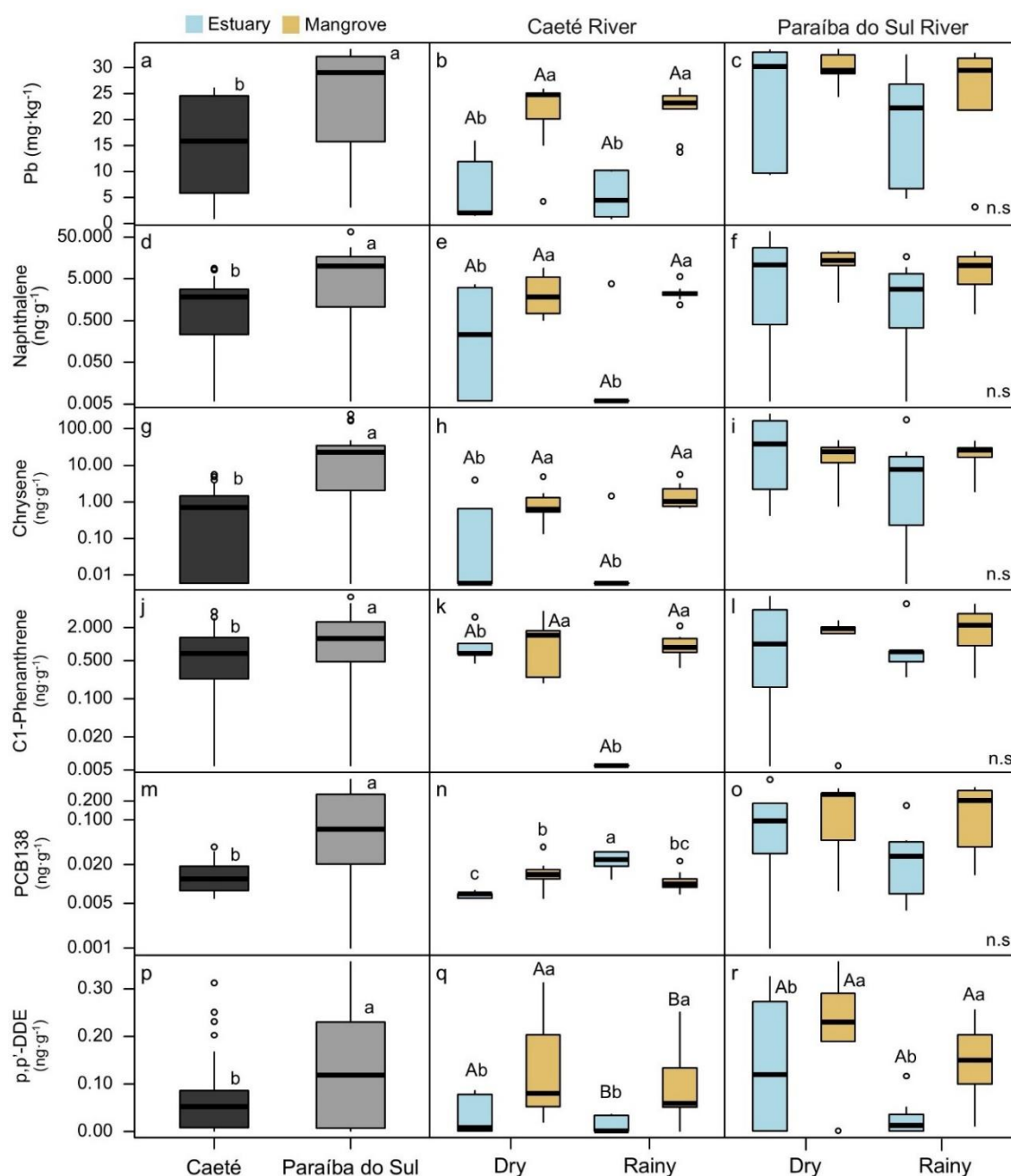


Fig.: 2 Concentrations of potential contaminants in surface sediment and soil from CR and PSR basins. Comparisons are shown by basin (1st column) and season (2nd and 3rd column). Lowercase and uppercase letters indicate significant differences ($p < 0.05$) among environments and seasons, respectively. When interactions were significant, lowercase letters indicate differences among factor combinations. n.s: not significant. When necessary, Y-axis was log-transformed for better visualization.

Reproductive Toxicity of Sediment-exposed Copepods

Physicochemical parameters did not show noticeable variations across basins and remained stable during the assay (Table S1), with values always above those recommended by Lotufo and Abessa (2002) and ABNT NBR 16723. Seasonality had no significant effect on offspring production and was not retained in the final model. The Basin $\times$ Environment interaction was significant ($p < 0.01$), indicating that the effect of sampling environment on reproduction differed between basins, and post-hoc comparisons were therefore performed across all Basin $\times$ Environment combinations. In CR basin, no significant differences were detected among control, estuarine and mangrove samples, although a trend of decreasing offspring production from control to mangrove sediments was evident (mean = 55.8, 38.8 and 22.9, respectively; Fig. 3a). This pattern was also observed in the PSR basin (mean = 118.0, 57.2, and 16.9 offspring, respectively; Fig. 3b), where mangrove samples showed a marked and significant ($p < 0.05$) reduction in reproductive output compared to estuarine (2.1-fold lower) and control samples (7.0-fold lower).

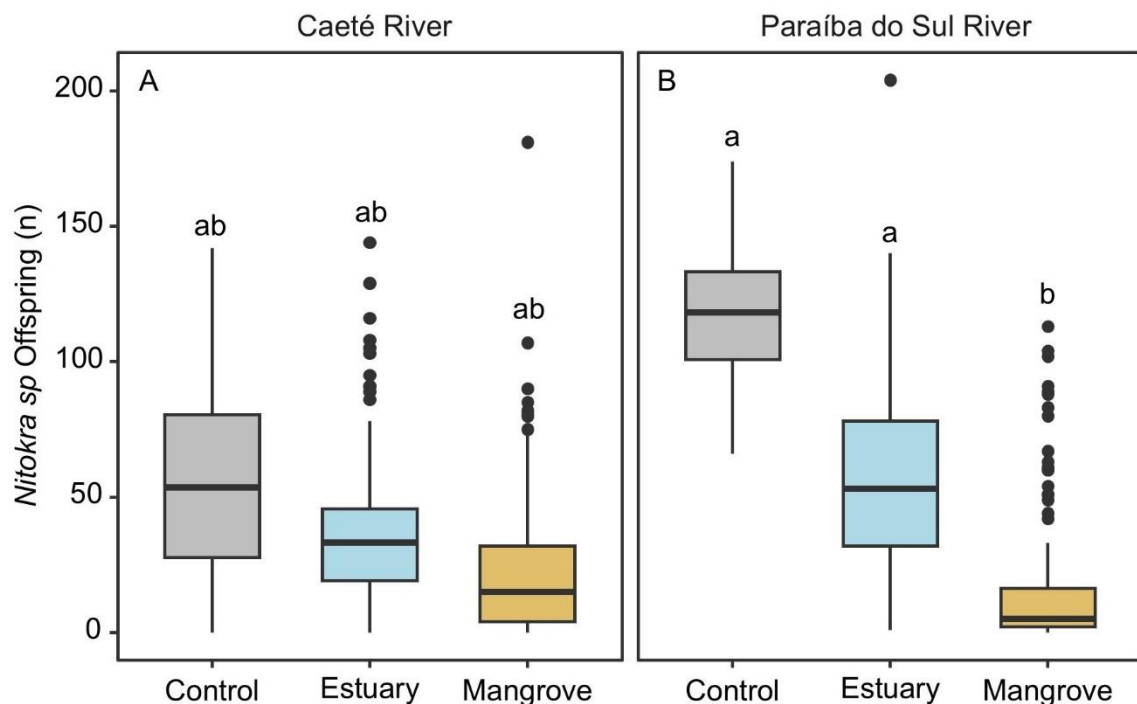


Fig.: 3 Offspring production of *Nitokra* sp. across sampling environments in the Caeté River (a) and Paraíba do Sul River (b) basins. Boxplots represent observed data. Letters denote significant differences among all Basin $\times$ Environment combinations based on pairwise contrasts of estimated marginal means (generalized Poisson GLMM, Tukey-adjusted, $\alpha = 0.05$).

Contaminant Mixture Effects on Reproductive Output

Given the inherent limitations of mixture models under small sample sizes ($n = 53$), statistical significance was not used as the sole criterion for interpreting results. Instead, emphasis was placed on the direction and magnitude of the mixture effect (Ψ), with p -values treated as supplementary evidence of signal strength rather than definitive thresholds.

Overall, results revealed predominantly negative associations between contaminant mixtures and offspring production, with metals representing the only class associated with a positive effect (Fig. 4). The simultaneous one-quartile increase across the 6 PCBs (138, 101, 44, 66, 52, 18) was associated with the strongest and statistically significant reduction in offspring production (43.3%), followed by the four OCPs (γ -HCH, p,p' -DDE, p,p' -DDD, p,p' -DDT; 39.3%). Although not statistically significant, HMW-, LMW-, and alkylated PAHs also showed negative associations, with estimated reductions of 36.1, 25.1, and 5.1%, respectively. Metals (Cd, Ca, Cr, Cu, Ni, Pb and Zn) and As were the only class with a positive association with reproductive output (+23.6%). Sensitivity analyses revealed that this effect was largely driven by Ca, as its removal from the mixture reduced the positive estimate by approximately 17 percentage points, with wide and overlapping confidence intervals in both models.

Importantly, the cross-class mixture estimate integrates the simultaneous contribution of all contaminant groups, whereas the class-specific estimates isolate the contribution of each chemical class independently. Therefore, Fig. 4 identifies which contaminant classes are associated with overall reproductive decline, while Fig. 5 decomposes these class-level effects to identify the compounds primarily responsible for the observed associations.

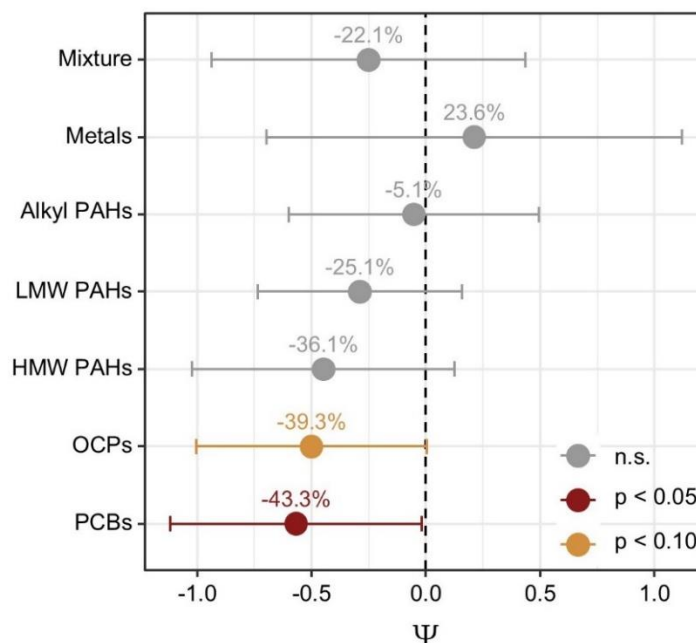


Fig.: 4 Mixture effect estimates (Ψ) for each contaminant class and the cross-class mixture model obtained by quantile g-computation. Points represent Ψ estimates with 95% confidence intervals. Percentages correspond to the estimated change in relative reproductive output. Colors denote significance levels. Color denotes significance levels: red, $p < 0.05$; orange, $p < 0.10$; gray, not significant.

Individual proportional weights (w_j) and Elastic Net coefficients (β) were then used as complementary indicators of contaminant importance and interpreted as directional metrics within both the cross-class mixture and the individual class-specific mixtures (*i.e.*, PCBs, OCPs, LMW-, HMW- and alkylated PAHs, metals and As), with Elastic Net coefficients given priority in cases of directional discordance between methods. This interpretation was conducted hierarchically at two levels: (i) across contaminant classes, to determine which classes contributed most strongly to the overall cross-class mixture effect, and (ii) within each class, to identify the individual compounds driving the class-level associations.

At the cross-class level, PCBs and HMW-PAHs emerged as the most consistent negative contributors to the overall mixture effect, while alkylated PAHs were identified as candidate contributors to reduce reproductive output. Metals and As, in contrast, contributed positively to reproductive output, consistent with the dominant influence of Ca observed in sensitivity analyses. Within the metals and As class, Pb was identified as a robust negative driver, whereas Ca showed the opposite pattern, acting as a

candidate contributor to increased reproductive output. In LMW- and HMW-PAHs, Naphthalene and Chrysene were identified as robust drivers, while C₁-Phenanthrene and C₂-Naphthalene were identified as probable drivers within alkylated PAHs. Within PCBs, representatives from three chlorination levels were identified as drivers, with PCB138 (hexa-CB) classified as robust and PCB44 and PCB101 (tetra- and penta-CBs) as probable. Among OCPs, both DDT metabolites showed concordant negative associations, with *p,p'*-DDE classified as a robust driver and *p,p'*-DDD as a probable driver of reproductive toxicity.

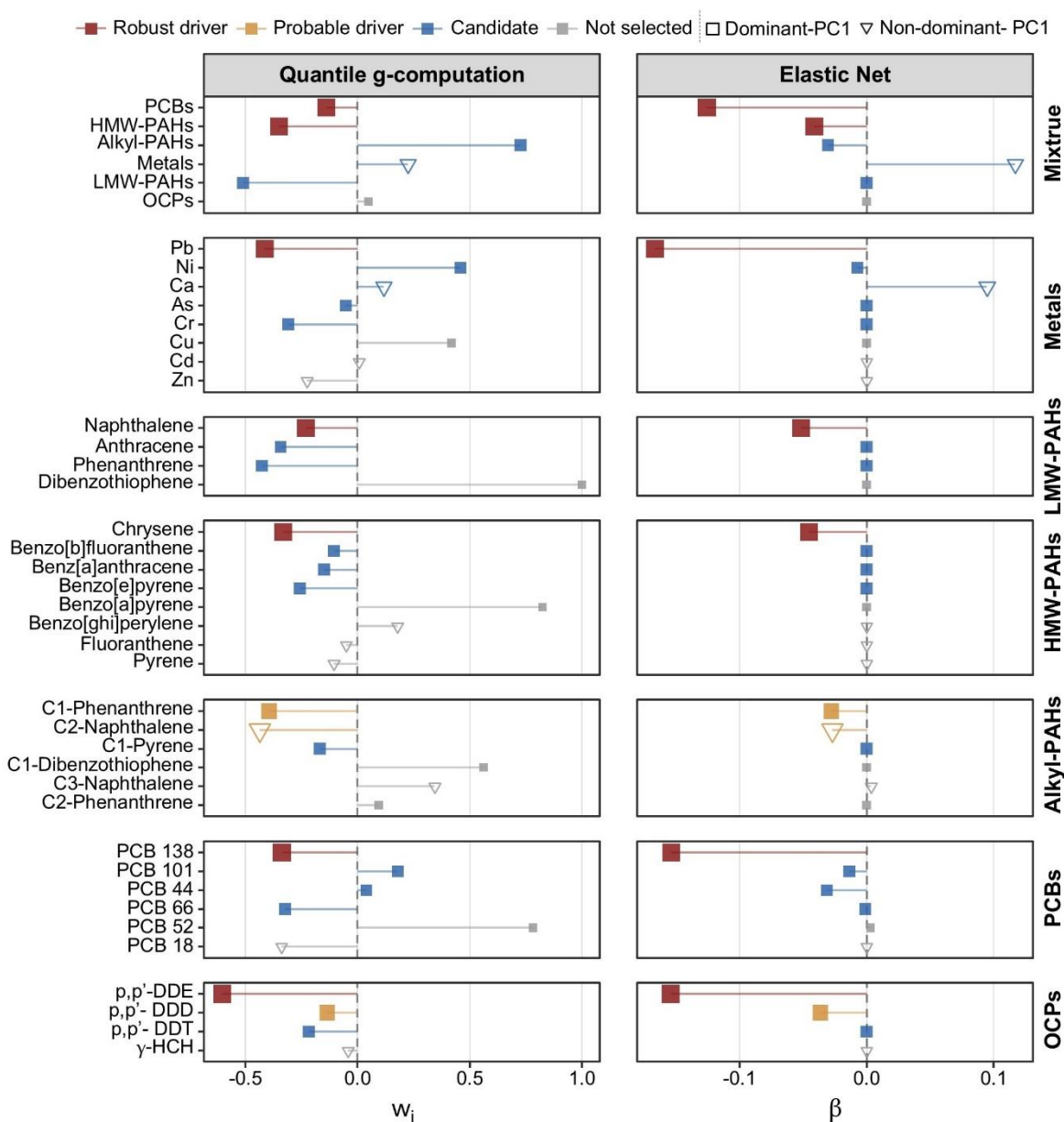


Fig.: 5 Convergence analysis integrating quantile g-computation, Elastic Net regression, and PCA for the identification of individual toxicity drivers within each contaminant class. The left panel displays the individual proportional weights (w_j) from adjusted quantile g-computation models, representing the relative contribution of each

compound to the class-level mixture effect (Ψ). The right panel displays the mean penalized regression coefficients from Elastic Net representing the expected change in $\ln(\text{offspring})$ per unit change in $\ln(\text{concentration})$.

Linking Contaminant Mixture to Reproductive Toxicity

The lower offspring production observed in mangrove environments, particularly in the PSR basin, is consistent with the elevated contaminant concentrations recorded in these sediments. The linear model combining geochemical and spatial confounders with the three classes showing the strongest negative associations with reproductive output (PCBs, OCPs, and HMW-PAHs) explained 40% of the variance in offspring production ($R^2 \text{ adj} = 0.40$, $p < 0.001$), with the Basin $\times$ Environment interaction reflecting the markedly lower reproductive output observed in PSR mangrove sediments. However, no individual class contributed significantly in isolation. In this sense, it was only when contaminants were evaluated as simultaneous quartile shifts across all compounds within a class that especially PCBs and OCPs emerged as negative influences on reproduction output, suggesting that the joint increase of these contaminants, rather than the absolute concentration of any single compound, underlies the observed toxicity.

Discussion

The consistent decreasing gradient in offspring production from control to estuarine to mangrove sediments, observed across both basins, provides evidence that sediment-associated contaminants in tropical estuaries negatively impair the reproduction of benthic copepods. Therefore, this ecosystem serves as a habitat for marine vertebrates and invertebrates during their reproductive phase, this evidence highlights that the presence of these contaminants poses significant risks during the early reproductive stages of numerous species. Such systems, particularly mangroves and low hydrodynamic energy sectors, favor the deposition of fine-grained, OM-rich sediments (Bayen, 2012; Mbusnum et al., 2020; Sartori et al., 2026). These zones act as preferential contaminants sinks, whose degree of accumulation varies with the proximity and intensity of urban, industrial, and agricultural pressures within the drainage basins (Barhoumi et al., 2019; Silva et al., 2023; Gurgatz et al., 2023; Sartori et al., 2026). This gradient is reflected in the present study data, where offspring production in PSR mangrove sediments was reduced nearly 7-fold relative to controls

(Fig. 3), alongside the highest median concentrations recorded for approximately 50, 100, 100, 80, 80, and 50% of metals, LMW-PAHs, HMW-PAHs, alkylated PAHs, PCBs, and OCPs, respectively (Figs. S1–S6). Although not statistically significant, the same decreasing pattern was observed in the CR basin, where the second highest median concentrations for most contaminant classes were also recorded.

According to Sartori et al. (2026), the CR and PSR basins reflect distinct trajectories of economic development and land occupation in Brazil, resulting in different levels and types of contamination between regions. The PSR basin drains one of the most industrially dense zones in Brazil, accumulating decades of inadequate wastewater discharge and intensive agrochemical use (Pfeiffer et al., 1986; AGEVAP, 2020; Souza et al., 2026), reflected in the elevated PTEC concentrations typically associated with urban-industrial and agricultural sources, such as PAH (Fig. S2–S4), medium- to high-chlorination PCBs (e.g., PCB101, PCB138; Fig. S5), DDT metabolites (*p,p'*-DDE, *p,p'*-DDD; Fig. S6), and metals associated with industrial and agricultural sources (e.g., Cu, Pb, Zn, and Ni; Fig. S1), as well as the lowest offspring production recorded (Fig. 3). Furthermore, the absence of seasonal differences in PTEC concentrations reinforces a legacy contamination pattern rather than pulses driven by seasonal runoff. The CR basin, in turn, faces more diffuse environmental pressures, primarily associated with inadequate sanitation, small-scale agricultural activities, and mining (Gorayeb et al., 2009, 2011; Sartori et al., 2026). Although contaminant concentrations were consistently lower than in the PSR basin, CR mangrove soils presented significantly higher concentrations than estuarine sediments for most classes (Fig. S1–S6), alongside with lower offspring production, suggesting that even under moderate anthropogenic pressure, mangroves act as effective sinks for PTEC.

The relatively high contaminant concentrations in mangroves are consistent with the higher fine-grained sediment and OC content recorded in these environments relative to estuaries (OC: 3.9- and 4.7-fold higher in CR and PSR mangroves, respectively; Sartori et al., 2026). These sedimentary properties play a central role in the retention, distribution, and fate of POPs and metals. Due to their hydrophobic nature, organic compounds exhibit strong affinity for OM-rich particles, partitioning from the aqueous to the particulate phase and thereby reducing their bioavailability in the water column (Nybom et al., 2024). This behavior is reflected in the present results, as organic contaminants were positively correlated with OC content within the same environment (Sartori et al., 2026). Although this partitioning reduces immediate toxicity,

it preserves contaminants in the environment by protecting them from biological and chemical degradation (Jones and de Voogt, 1999; Nybom et al., 2024). For metals, Fe plays an additional geochemical role, as under oxidizing conditions it forms oxyhydroxides that act as important adsorptive phases through coprecipitation and surface adsorption. Under reducing conditions, the dissolution of these oxyhydroxides may release metals into porewater, although the presence of sulfides tends to immobilize them as insoluble forms (Lacerda et al., 1993; Shi et al., 2021; Silva et al., 2023). Critical remobilization occurs when anoxic sediments are disturbed and reoxidized by dredging, bioturbation, or tidal cycles, causing sulfide oxidation and physical resuspension of surface sediment layers, releasing metals and organic compounds back into the water column where they become bioavailable to benthic organisms or transportable to adjacent estuarine systems (Lacerda et al., 1993; Monte et al., 2025).

The PTEC concentrations recorded in the present study are comparable to those reported in other aquatic systems under strong urban-industrial influence, particularly for the compounds selected by the mixture effect model. Pb, for example, presented mean concentrations of 15.07 and 23.74 mg·kg⁻¹ in the CR and PSR basins, respectively, values within the range reported for urbanized and industrialized Brazilian estuaries, such as the Serinhém estuary (26.50 mg·kg⁻¹; Silva et al., 2023), and the Santa Maria da Vitória estuary (16.60 mg·kg⁻¹; Sartori et al., 2023), and approaching those of severely contaminated systems, such as the Doce River, Brazil (20.80 mg·kg⁻¹; Sartori et al., 2023), and the Gao-Ping River, Taiwan (28.20 mg·kg⁻¹; Doong et al., 2008). For PAHs, the individual concentrations of the selected compounds in the PSR basin (*i.e.*, Naphthalene: 11.95 ng·g⁻¹; Fluoranthene: 84.71 ng·g⁻¹ e C₂-Naphthalene: 17.83 ng·g⁻¹) already exceed, individually, the class mean concentrations of LMW-PAHs (5.50 ng·g⁻¹), HMW-PAHs (11.20 ng·g⁻¹) and alkylated -PAHs (13.20 ng·g⁻¹) reported for Paranaguá Bay, one of Brazil's major port complexes (Gurgatz et al., 2023). Likewise, the mean concentrations of LMW-, HMW-, and alkylated PAHs in the present study were of the same order of magnitude as those reported for the São Paulo coastal region under intense anthropogenic pressure (Martins et al., 2023). In the CR basin, the same compounds were 2.6-, 6.3-, and 2.4-fold lower than these class sums, respectively when compared with the same study. The *p,p'*-DDE metabolite, in turn, presented concentrations exceeding the mean values reported for Paranaguá Bay (0.04 ng·g⁻¹), in both the CR (0.07 ng·g⁻¹) and PSR (0.13 ng·g⁻¹; Souza et al., 2018a)

basins, and within the range observed in the Pearl River, China ($0.01 - 1.81 \text{ ng}\cdot\text{g}^{-1}$; Tang et al., 2020). Although PCB concentrations recorded in the present study (sum of the six selected congeners $< 0.1 \text{ ng}\cdot\text{g}^{-1}$) remain well below the means reported for severely contaminated systems such as the Santos Estuary, Brazil ($3.19 \text{ ng}\cdot\text{g}^{-1}$; Souza et al., 2018b), PCBs emerged as the class most strongly and negatively associated with reproductive output in *Nitokra sp.*, reinforcing the argument that toxicological effects arise from simultaneous exposure to multiple PTECs rather than from the absolute concentration of any single compound.

Despite these similarities with recognized impacted systems, most compounds identified as drivers of reproductive toxicity presented concentrations below sediment quality guidelines, expressed as Threshold Effect Level (TEL) and Probable Effect Level (PEL), reference concentrations below and above which adverse effects are, respectively, rarely or frequently expected (Buchman, 2008). DDT metabolites and PCBs remained well below TELs, as did Pb and most LMW-PAHs. Only in the PSR basin did mean concentrations of As ($7.98 \text{ mg}\cdot\text{kg}^{-1}$) and Cr ($60.25 \text{ mg}\cdot\text{kg}^{-1}$), and some HMW-PAHs (Pyrene: $80.23 \text{ ng}\cdot\text{g}^{-1}$, Benz[a]anthracene: $46.91 \text{ ng}\cdot\text{g}^{-1}$, Benzo[a]pyrene: $48.69 \text{ ng}\cdot\text{g}^{-1}$) approach or exceed TELs for fresh and marine sediments. However, independent of these results, one must consider the synergistic effects of these contaminants in causing adverse effects on the species inhabiting this ecosystem at different stages of their reproductive life cycle.

In this context, the main contribution of the present study was to demonstrate that reproductive toxicity in the copepod *Nitokra sp.* was associated with simultaneous exposure to multiple PTECs at concentrations that, individually, remained mostly below sediment quality guidelines. Since models were adjusted for geochemical and spatial confounders, the mixture effect estimates (Ψ) reflect associations independent of these contextual conditions, suggesting the observed effects result predominantly from the combined action of contaminants rather than from any single compound. Results indicated that the main drivers of offspring production reduction were, in order of importance, PCBs > OCPs > HMW- > LMW- > alkylated -PAHs (Fig. 4), reinforcing the dominant role of POP in reproductive toxicity. In contrast, metals and As did not show a negative association as a class, exhibiting a positive relationship driven primarily by Ca concentrations (Figs. 4 and 5), an essential element involved in crustacean physiological processes including exoskeleton formation, ecdysis regulation, and gonadal development (Huang et al., 2021; Jiang et al., 2025). This result suggests that

the response of the element class reflects the combined influence of essential and potentially toxic elements, contrasting with the predominantly xenobiotic nature of the organic contaminant classes. The high sensitivity of *Nitokra* sp. to sediment-associated contaminants, together with its ecological relevance, and widespread occurrence in tropical benthic environments, reinforces its suitability as a biological indicator for assessing chronic toxicity in tropical coastal sediments (Lotufo and Abessa, 2002; Ward et al., 2011). Collectively, these results suggest that the observed reproductive response cannot be explained solely by exceedance of individual toxicity thresholds, but rather by chronic exposure to complex contaminant mixtures, even at levels considered safe under conventional sediment quality criteria.

In addition to being highly stable, resistant to decomposition, and poorly soluble in water, POPs have a strong affinity for fatty tissues, bioaccumulating in cellular lipid layers and conferring their highly toxic characteristics (Jones and de Voogt, 1999; Kumar et al., 2022). Along the food chain, these compounds biomagnify, reaching increasing concentrations at higher trophic levels (Fremlin et al., 2020; Kumar et al., 2022). Copepods are particularly vulnerable to this process, as they strongly depend on lipids as energy reserves (Werbrouck et al., 2016) and feed on organic particles and suspended detritus, directly ingesting these contaminants (Koski and Lombard, 2022). The evidence triangulation reinforces PCBs and OCPs as the primary drivers of the observed reduction in copepod offspring production. This chronic exposure has direct implications for reproductive toxicity, as these compounds are recognized endocrine disruptors (Fremlin et al., 2020; Kumar et al., 2022) that, in invertebrates, compromise ecdysteroid signaling, hormones controlling ecdysis, development, and spawning (Soin et al., 2009). As first-order consumers, key vectors of vertical carbon flux by processing OM before it settles, and vehicles of contaminant transfer to higher trophic levels (Koski and Lombard, 2022), impairments in their reproduction represent a risk to the entire aquatic food web.

Beyond identifying contaminant classes associated with reproductive toxicity, the principal contribution of the present study lies in demonstrating a framework for sediment quality assessment that integrates ecotoxicological responses with contaminant mixture modeling. Current sediment assessments remain largely based on comparisons between measured contaminant concentrations and sediment quality guidelines, implicitly assuming that contaminants act independently. However, the present results demonstrate that biologically relevant effects can occur even when

most contaminants remain below their respective guideline values, becoming evident only when contaminants are evaluated as complex mixtures. By integrating biological responses with mixture modeling, this framework provides an additional line of evidence capable of identifying not only contaminated sediments, but also the contaminant classes and individual compounds most strongly associated with the observed biological impairment. Rather than replacing existing sediment quality guidelines, this approach should be viewed as complementary, improving contaminant prioritization under realistic multiple-stressor conditions. Incorporating mixture-based analyses into sediment monitoring programs therefore represents a natural step toward more ecologically relevant sediment assessment. As complementary lines of evidence, such as contaminant bioavailability, biomarkers of exposure and effect, species sensitivity, and ecological responses become increasingly available, this framework could evolve from identifying toxicity drivers toward supporting more comprehensive ecological risk assessment.

Some limitations should be acknowledged: the small sample size makes comparison among basin and their respective environment types impossible, constrained statistical power, and individual mixture weights were therefore interpreted as directional indicators of association rather than precise quantitative estimates. Furthermore, inorganic elements were modeled as a single exposure class to preserve model stability and avoid further increasing model complexity relative to the available sample size. Although Ca is an essential macronutrient rather than a toxic trace metal, sensitivity analyses demonstrated that its inclusion influenced only the magnitude of effect. Future studies should expand spatial and temporal coverage, incorporate larger datasets encompassing broader environmental gradients, and evaluate whether the identified mixture effects remain consistent across distinct estuarine settings and biological endpoints. Nevertheless, the consistency of results across three analytically independent methods, combined with model adjustment for major geochemical and spatial covariates, strengthens confidence in the identified drivers and suggests that the findings may extend to others tropical estuarine systems and sedimentary characteristics. Taken together, the observed reproductive impairment cannot be explained solely by the exceedance of individual toxicity thresholds but rather reflects the cumulative effects of chronic exposure to mixtures, even at concentrations individually considered safe under conventional sediment quality criteria.

Conclusion

The present study demonstrates that reproductive toxicity is driven by simultaneous exposure to complex contaminant mixtures in tropical estuarine sediments, even when individual compound concentrations remain below conventional sediment quality guidelines. The consistent decreasing gradient in offspring production from control to estuarine and mangrove environments across both basins reflects the role of mangroves as preferential sinks for PTECs, where contaminant accumulation is favored by elevated organic carbon content and fine sediment fractions. A hierarchical analytical pipeline integrating quantile g-computation, Elastic Net regression, and PCA consistently identified PCBs and OCPs as the primary drivers of reproductive impairment, followed by HMW- and LMW-PAHs, while the positive association observed for inorganic elements was largely explained by Ca. The contrasting contamination histories of the PSR and CR basins further demonstrate how land-use trajectories shape contaminant mixtures and their biological consequences. Collectively, these findings show that evaluating contaminants as complex mixtures, rather than as isolated compounds, provides a more ecologically meaningful interpretation of sediment quality. By integrating ecotoxicological responses with mixture modeling, the proposed framework offers a complementary strategy for contaminant prioritization and represents a step toward more effect-based sediment monitoring and ecological risk assessment.

CRedit authorship contribution statement

Échily Sartori: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization.

Cristiane dos Santos Vergilio: Conceptualization, Writing – review & editing, Supervision.

Jessica Dutra Ferreira: Methodology, Review & Editing

Ingrid Ferreira dos Santos: Methodology, Review & Editing

Ana Luiza Gonçalves Sepulcro: Methodology, Review & Editing

Diego Lacerda: Writing – review & editing

Luiza Silva do Nascimento: Investigation

Pedro Vianna Gatts: Investigation, Review & Editing

Jamile Tamer Nasser: Methodology, Validation, Data curation

César de C. Martins: Validation, Review & Editing

Erika Kurahashi: Methodology, Validation, Data curation, Review & Editing

Lukas Klose: Methodology, Validation, Data curation, Review & Editing

Andrea Koschinsky: Validation, Resources, Review & Editing

Nils E. Asp: Investigation, Review & Editing

Carlos Eduardo de Rezende: Conceptualization, Validation, Investigation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgments

We thank the Environmental Science Laboratory (Laboratório de Ciências Ambientais – LCA) of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, the Marine Organic Chemistry Laboratory of the Universidade de São Paulo, the Coastal Geology Laboratory of the Universidade Federal do Pará and the OceanLab from Constructor University for providing infrastructure for sampling collection and further analysis.

Funding statement

É. Sartori received grant from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (88887.824229/2023-00) - Finance Code 001. É. Sartori and L. S. do Nascimento received scholarship from the CAPES/DAAD Probral Program, which supports international academic cooperation between Brazil and Germany (88887.989816/2024-00 and 88887.985811/2024-00). C.C. Martins thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant code 310850/2020-7). Carlos E. de Rezende received grant from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; Grant Code E-26/203.950/2024) and CNPq (Grant Code 310.260/2023-0 and 409.378/2022-4) and P. V. Gatts received grant from FAPERJ (Grant Code E-26/204.508/2021 and E-26/204.509/2021).

Declaration of interest statement

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.

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

All the text of the manuscript was initially written without AI. AI was used to text improve and correct english formulations. AI was also used for construction of R codes.

Data availability

Data will be made available on request.

References

- [1] Abdala, G., Saraiva, N., & Wesley, F. (2012). Plano de Manejo da Reserva Extrativista Caeté-Taperaçu – Volume I – Diagnóstico da Unidade de Conservação. Brasília: ICMBio, 109 p.
- [2] ABNT – Associação Brasileira de Normas Técnicas. (2021). Ecotoxicologia aquática – Método de ensaio com copépodos marinhos e estuarinos (Copepoda, Crustacea) (ABNT NBR 16723:2021).
- [3] AGEVAP – Associação Pró-Gestão das Águas da Bacia Hidrográfica do Rio Paraíba do Sul. (2020). Relatório sobre cenário ambiental 2020. 171 p. <https://www.ceivap.org.br/>
- [4] Altman, N., & Krzywinski, M. (2016). Regression diagnostics. *Nature Methods*, 13(5), 385–387. <https://doi.org/10.1038/nmeth.385>
- [5] Asp, N. E., Gomes, V. J. C., Schettini, C. A. F., Souza-Filho, P. W. M., Siegle, E., Ogston, A. S., Nittrouer, C. A., Silva, J. N. S., Nascimento, W. R., Jr., Souza, S. R., Pereira, L. C. C., & Queiroz, M. C. (2018). Sediment dynamics of a tropical tide-dominated estuary: Turbidity maximum, mangroves and the role of the Amazon River sediment load. *Estuarine, Coastal and Shelf Science*, 214, 10–24. <https://doi.org/10.1016/j.ecss.2018.09.004>
- [6] Barhoumi, B., Beldean-Galea, M. S., Al-Rawabdeh, A. M., Roba, C., Martonos, I. M., Bălc, R., Kahlaoui, M., Touil, S., Tedetti, M., Driss, M. R., & Baciú, C. (2019). Occurrence, distribution and ecological risk of trace metals and organic pollutants in surface sediments from a Southeastern European river (Someșu Mic River, Romania). *Science of The Total Environment*, 660, 660–676. <https://doi.org/10.1016/j.scitotenv.2018.12.428>

- [7] Bayen, S. (2012). Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: A review. *Environment International*, 48, 84–101. <https://doi.org/10.1016/j.envint.2012.07.008>
- [8] Bernini, E., & Rezende, C. E. (2011). Vegetation structure in a mangrove forest in Southeastern Brazil. *Pan-American Journal of Aquatic Sciences*, 6(3), 193–209.
- [9] Brazil. (1981). Portaria Interministerial nº 19. Ministérios do Interior, da Indústria e Comércio e de Minas e Energia. *Diário Oficial da União*. <https://faolex.fao.org/docs/pdf/bra15389.pdf>
- [10] Brazil. (1985). Portaria nº 329. Ministério da Agricultura. *Diário Oficial da União*. https://bvsms.saude.gov.br/bvs/saudelegis/mapa_gm/1985/prt0329_02_09_1985.html
- [11] Brazil. (2009). Lei nº 11.936, de 14 de maio de 2009. *Diário Oficial da União*. https://planalto.gov.br/ccivil_03/Ato2007-2010/2009/Lei/L11936.htm
- [12] Brooks, M. E., Kasper, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9(2), 378–400. <https://doi.org/10.32614/RJ-2017-066>
- [13] Buchman, M. F. (2008). NOAA screening quick reference tables (NOAA OR&R Report 08-1). Seattle, WA: Office of Response and Restoration Division, National Oceanic and Atmospheric Administration, 34 p.
- [14] Camargo, M. G. (2006). Sysgran: Um sistema de código aberto para análises granulométricas do sedimento. *Revista Brasileira de Geociências*, 36(2), 371–378.
- [15] Çelebi, A., Canlı, O., Güzel, B., & Çetintürk, K. (2024). Ecotoxicological risk assessments and components of persistent organic pollutants and metals in the historical settlement area (Iznik (Nicea) lake) large water resource sediments. *Marine Pollution Bulletin*, 202, 116339. <https://doi.org/10.1016/j.marpolbul.2024.116339>
- [16] Dittmar, T., Lara, R. J., & Kattner, G. (2001). River or mangrove? Tracing major organic matter sources in tropical Brazilian coastal waters. *Marine Chemistry*, 73(3–4), 253–271. [https://doi.org/10.1016/S0304-4203\(00\)00110-9](https://doi.org/10.1016/S0304-4203(00)00110-9)

- [17] Doong, R., Lee, S., Lee, C., Sun, Y., & Wu, S. (2008). Characterization and composition of heavy metals and persistent organic pollutants in water and estuarine sediments from Gao-ping River, Taiwan. *Marine Pollution Bulletin*, 57(6–12), 846–857. <https://doi.org/10.1016/j.marpolbul.2007.12.015>
- [18] Folk, R. L., & Ward, W. C. (1957). Brazos River bar (Texas): A study in the significance of grain size parameters. *Journal of Sedimentary Research*, 27(1), 3–26. <https://doi.org/10.1306/74D70646-2B21-11D7-8648000102C1865D>
- [19] Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>
- [20] Fremlin, K. M., Elliott, J. E., Green, D. J., Drouillard, K. G., Harner, T., Eng, A., & Gobas, F. A. P. C. (2020). Trophic magnification of legacy persistent organic pollutants in an urban terrestrial food web. *Science of The Total Environment*, 714, 136746. <https://doi.org/10.1016/j.scitotenv.2020.136746>
- [21] Friedman, J., Hastie, T., & Tibshirani, R. (2010). Regularization paths for generalized linear models via coordinate descent. *Journal of Statistical Software*, 33(1), 1–22. <https://doi.org/10.18637/jss.v033.i01>
- [22] Gorayeb, A., Lombardo, M. A., & Pereira, L. C. C. (2009). Condições ambientais em áreas urbanas da bacia hidrográfica do rio Caeté – Amazônia Oriental, Brasil. *Revista de Gestão Costeira Integrada*, 9(2), 59–70. <https://doi.org/10.5894/rgci117>
- [23] Gorayeb, A., Lombardo, M. A., & Pereira, L. C. C. (2011). Natural conditions and environmental impacts in a coastal hydrographic basin in the Brazilian Amazon. *Journal of Coastal Research*, 64. <https://www.jstor.org/stable/26482393>
- [24] Graves, S., Piepho, H., & Dorai-Raj, S. (2024). multcompView: Visualizations of paired comparisons. R package version 0.1-10. <https://CRAN.R-project.org/package=multcompView>
- [25] Gu, Y.-G., Gao, Y.-P., Chen, F., Huang, H.-H., Yu, S.-H., Jordan, R. W., & Jiang, S.-J. (2022). Risk assessment of heavy metal and pesticide mixtures in aquatic biota using the DGT technique in sediments. *Water Research*, 224, 119108. <https://doi.org/10.1016/j.watres.2022.119108>
- [26] Gurgatz, B. M., Garcia, M. R., Cabral, A. C., de Souza, A. C., Nagai, R. H., Figueira, R. C. L., de Mahiques, M. M., & Martins, C. C. (2023). Polycyclic aromatic

hydrocarbons in a Natural Heritage Estuary influenced by anthropogenic activities in the South Atlantic: Integrating multiple source apportionment approaches. *Marine Pollution Bulletin*, 188, 114678. <https://doi.org/10.1016/j.marpolbul.2023.114678>

[27] Hartig, F. (2024). DHARMA: Residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.7. <https://CRAN.R-project.org/package=DHARMA>

[28] Huang, J., Xu, X., Li, D., Sun, Y., Gu, L., Zhang, L., Lyu, K., & Yang, Z. (2021). Decreased calcium concentration interferes with life history defense strategies of *Ceriodaphnia cornuta* in response to fish kairomone. *Limnology and Oceanography*, 66(8), 3237–3252. <https://doi.org/10.1002/lno.11876>

[29] Jiang, X., Ou, F., Jia, T., Guo, H., Liu, P., Tian, W., Wu, S., Chen, S., Feng, W., & Zhang, W. (2025). Effects of increased calcium, magnesium, and potassium ion concentrations on survival conditions, growth performance, and physiological parameters in sea urchin (*Strongylocentrotus intermedius*). *Biology*, 14(8), 1046. <https://doi.org/10.3390/biology14081046>

[30] Jones, K. C., & de Voogt, P. (1999). Persistent organic pollutants (POPs): State of the science. *Environmental Pollution*, 100(1–3), 209–221. [https://doi.org/10.1016/S0269-7491\(99\)00098-6](https://doi.org/10.1016/S0269-7491(99)00098-6)

[31] Kay, M., Elkin, L., Higgins, J., & Wobbrock, J. (2025). ARTool: Aligned rank transform for nonparametric factorial ANOVAs. R package version 0.11.2. <https://doi.org/10.5281/zenodo.594511>

[32] Keil, A. (2025). ggcomp: Quantile g-computation. R package version 2.18.7. <https://CRAN.R-project.org/package=ggcomp>

[33] Keil, A. P., Buckley, J. P., O'Brien, K. M., Ferguson, K. K., Zhao, S., & White, A. J. (2020). A quantile-based g-computation approach to addressing the effects of exposure mixtures. *Environmental Health Perspectives*, 128(4). <https://doi.org/10.1289/EHP5838>

[34] Koski, M., & Lombard, F. (2022). Functional responses of aggregate-colonizing copepods. *Limnology and Oceanography*, 67(9), 2059–2072. <https://doi.org/10.1002/lno.12187>

- [35] Kumar, J. A., Krithiga, T., Sathish, S., Renita, A. A., Prabu, D., Lokesh, S., Geetha, R., Namasivayam, S. K. R., & Sillanpaa, M. (2022). Persistent organic pollutants in water resources: Fate, occurrence, characterization and risk analysis. *Science of The Total Environment*, 831, 154808. <https://doi.org/10.1016/j.scitotenv.2022.154808>
- [36] Lacerda, L. D., Carvalho, C. E. V., Tanizaki, K. F., Ovalle, A. R. C., & Rezende, C. E. (1993). The biogeochemistry and trace metals distribution of mangrove rhizospheres. *Biotropica*, 25(3), 252. <https://doi.org/10.2307/2388783>
- [37] Lenth, R. (2024). emmeans: Estimated marginal means, aka least-squares means. R package version 1.10.3. <https://CRAN.R-project.org/package=emmeans>
- [38] Lotufo, G., & Abessa, D. (2002). Testes de toxicidade com sedimento total e água intersticial estuarinos utilizando copépodes bentônicos. In *Métodos em Ecotoxicologia Marinha: Aplicações no Brasil*.
- [39] Lugendo, B. R., & Kimirei, I. A. (2021). Anthropogenic nitrogen pollution in mangrove ecosystems along Dar es Salaam and Bagamoyo coasts in Tanzania. *Marine Pollution Bulletin*, 168, 112415. <https://doi.org/10.1016/j.marpolbul.2021.112415>
- [40] Martins, C. C., Moreira, L. B., Sutilli, M., & de Souza Abessa, D. M. (2023). Unraveling sources of hydrocarbons in subtropical estuaries with distinct degrees of protection in the Southwestern Atlantic Ocean, Brazil. *Marine Pollution Bulletin*, 195, 115499. <https://doi.org/10.1016/j.marpolbul.2023.115499>
- [41] Mbusnum, K. G., Malleret, L., Deschamps, P., Khabouchi, I., Asia, L., Lebarillier, S., Menot, G., Onguene, R., & Doumenq, P. (2020). Persistent organic pollutants in sediments of the Wouri Estuary Mangrove, Cameroon: Levels, patterns and ecotoxicological significance. *Marine Pollution Bulletin*, 160, 111542. <https://doi.org/10.1016/j.marpolbul.2020.111542>
- [42] Mendiburu, F. (2023). agricolae: Statistical procedures for agricultural research. R package version 1.3-7. <https://CRAN.R-project.org/package=agricolae>
- [43] Monte, C. N., Rodrigues, A. P. C., Campos, B. G., Moreira, L. B., Grebbe, B., Pauly, G. F. E., Cruz, A. C. F., Silva, L. I. D., Cordeiro, R. C., Abessa, D. M. S., & Machado, W. (2025). Influence of seasonality on the toxicity and metals bioavailability after the

resuspension of sediments from a tropical estuary. *Estuarine, Coastal and Shelf Science*, 315, 109167. <https://doi.org/10.1016/j.ecss.2025.109167>

[44] Nybom, I., van Grimbergen, J., Forsell, M., Mustajärvi, L., Martens, J., & Sobek, A. (2024). Water column organic carbon composition as driver for water-sediment fluxes of hazardous pollutants in a coastal environment. *Journal of Hazardous Materials*, 465, 133393. <https://doi.org/10.1016/j.jhazmat.2023.133393>

[45] Ogle, D. H., Doll, J. C., Wheeler, A. P., & Dinno, A. (2023). FSA: Simple fisheries stock assessment methods. R package version 0.9.5. <https://CRAN.R-project.org/package=FSA>

[46] Oliveira, A. H. B., Cavalcante, R. M., Duaví, W. C., Fernandes, G. M., Nascimento, R. F., Queiroz, M. E. L. R., & Mendonça, K. V. (2016). The legacy of organochlorine pesticide usage in a tropical semi-arid region (Jaguaribe River, Ceará, Brazil): Implications of the influence of sediment parameters on occurrence, distribution and fate. *Science of The Total Environment*, 542, 254–263. <https://doi.org/10.1016/j.scitotenv.2015.10.058>

[47] Oliveira, D. D., Souza-Santos, L. P., Silva, H. K. P., & Macedo, S. J. (2014). Toxicity of sediments from a mangrove forest patch in an urban area in Pernambuco (Brazil). *Ecotoxicology and Environmental Safety*, 104, 373–378. <https://doi.org/10.1016/j.ecoenv.2014.02.004>

[48] Pfeiffer, W. C., Fiszman, M., Malm, O., & Azcue, J. M. (1986). Heavy metal pollution in the Paraíba do Sul River. *The Science of the Total Environment*, 58, 73–79. [https://doi.org/10.1016/0048-9697\(86\)90077-X](https://doi.org/10.1016/0048-9697(86)90077-X)

[49] R Core Team. (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing. <https://www.R-project.org>

[50] Santos, F. R., Neves, P. A., Kim, B. S. M., Taniguchi, S., Lourenço, R. A., Timoszczuk, C. T., Sotão, B. M. T., Montone, R. C., Figueira, R. C. L., Mahiques, M. M., & Bicego, M. C. (2020). Organic contaminants and trace metals in the western South Atlantic upper continental margin: Anthropogenic influence on mud depocenters. *Marine Pollution Bulletin*, 154, 111087. <https://doi.org/10.1016/j.marpolbul.2020.111087>

- [51] Sartori, É., Vergilio, C. S., Gatts, P. V., de Oliveira, B. C. V., de Almeida, M. G., Thompson, F., & de Rezende, C. E. (2023). Trace metal concentration along the Brazilian coast: An assessment of the influence of the Doce River plume. *Marine Pollution Bulletin*, 188, 114640. <https://doi.org/10.1016/j.marpolbul.2023.114640>
- [52] Sartori, É., Vergilio, C. S., Serafim, T. S. G., Lacerda, D., do Nascimento, L. S., Gatts, P. V., Freitas, M., Nasser, J. T., de Almeida, M. G., Martins, C. C., Asp, N. E., & de Rezende, C. E. (2026). Land-use impacts on Amazon and Atlantic rainforest coastal ecosystems revealed by multiple biogeochemical markers. *Marine Pollution Bulletin*, 230, 119899. <https://doi.org/10.1016/j.marpolbul.2026.119899>
- [53] Shi, M., Min, X., Ke, Y., Lin, Z., Yang, Z., Wang, S., Peng, N., Yan, X., Luo, S., Wu, J., & Wei, Y. (2021). Recent progress in understanding the mechanism of heavy metals retention by iron (oxyhydr)oxides. *Science of The Total Environment*, 752, 141930. <https://doi.org/10.1016/j.scitotenv.2020.141930>
- [54] Silva, A. C. F. S., de Santana, C. O., dos Santos Vergilio, C., & de Jesus, T. B. (2023). Distribution, toxicity and bioaccumulation of trace metals in environmental matrices of an estuary in a protected area. *Marine Pollution Bulletin*, 191, 114928. <https://doi.org/10.1016/j.marpolbul.2023.114928>
- [55] Sisinno, C. L. S., & Oliveira-Filho, E. C. (2013). *Princípios de toxicologia ambiental: Conceitos e aplicações*. Interciência.
- [56] Soin, T., Verslycke, T., Janssen, C., & Smagghe, G. (2009). Ecdysteroids and their importance in endocrine disruption research. In *Ecdysone: Structures and Functions* (pp. 539–549). Springer Netherlands. https://doi.org/10.1007/978-1-4020-9112-4_22
- [57] Souza, A. C., Combi, T., da Silva, J., & Martins, C. C. (2018a). Occurrence of halogenated organic contaminants in estuarine sediments from a biosphere reserve in Southern Atlantic. *Marine Pollution Bulletin*, 133, 436–441. <https://doi.org/10.1016/j.marpolbul.2018.05.052>
- [58] Souza, A. C., Taniguchi, S., Figueira, R. C. L., Montone, R. C., Bicego, M. C., & Martins, C. C. (2018b). Historical records and spatial distribution of high hazard PCBs levels in sediments around a large South American industrial coastal area (Santos Estuary, Brazil). *Journal of Hazardous Materials*, 360, 428–435. <https://doi.org/10.1016/j.jhazmat.2018.08.041>

- [59] Souza, L. M. E., Vidal, L. O., Lima, V. S., Rezende, C. E., & Suzuki, M. S. (2026). Long-term variation of hydrochemical parameters in a medium-sized tropical river: Effects of a severe drought. *Journal of the Brazilian Chemical Society*. <https://doi.org/10.21577/0103-5053.20260033>
- [60] Tang, D., Liu, X., He, H., Cui, Z., Gan, H., & Xia, Z. (2020). Distribution, sources and ecological risks of organochlorine compounds (DDTs, HCHs and PCBs) in surface sediments from the Pearl River Estuary, China. *Marine Pollution Bulletin*, 152, 110942. <https://doi.org/10.1016/j.marpolbul.2020.110942>
- [61] UNEP. (2001). Stockholm Convention on Persistent Organic Pollutants (UNEP/POPS/CONF/2, as revised in 2025). Secretariat of the Stockholm Convention. <https://chm.pops.int/TheConvention/Overview>
- [62] Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed.). Springer.
- [63] Vergilio, C. S., Lacerda, D., de Oliveira, B. C. V., Sartori, É., Campos, G. M., Pereira, A. L. S., Aguiar, D. B., Souza, T. S., de Almeida, M. G., Thompson, F., & de Rezende, C. E. (2020). Metal concentrations and biological effects from one of the largest mining disasters in the world (Brumadinho, Minas Gerais, Brazil). *Scientific Reports*, 10(1). <https://doi.org/10.1038/s41598-020-62700-w>
- [64] Vergilio, C. S., Lacerda, D., Souza, T. S., de Oliveira, B. C. V., Fioresi, V. S., de Souza, V. V., da Rocha Rodrigues, G., Barbosa, M. K. A. M., Sartori, É., Rangel, T. P., de Almeida, D. Q. R., de Almeida, M. G., Thompson, F., & de Rezende, C. E. (2021). Immediate and long-term impacts of one of the worst mining tailing dam failures worldwide (Bento Rodrigues, Minas Gerais, Brazil). *Science of The Total Environment*, 756, 143697. <https://doi.org/10.1016/j.scitotenv.2020.143697>
- [65] Ward, D. J., Perez-Landa, V., Spadaro, D. A., Simpson, S. L., & Jolley, D. F. (2011). An assessment of three harpacticoid copepod species for use in ecotoxicological testing. *Archives of Environmental Contamination and Toxicology*, 61(3), 414–425. <https://doi.org/10.1007/s00244-011-9646-2>
- [66] Werbrouck, E., Van Gansbeke, D., Vanreusel, A., & De Troch, M. (2016). Temperature affects the use of storage fatty acids as energy source in a benthic copepod (*Platychelipus littoralis*, Harpacticoida). *PLOS ONE*, 11(3), e0151779. <https://doi.org/10.1371/journal.pone.0151779>

- [67] Yu, L., Liu, W., Wang, X., Ye, Z., Tan, Q., Qiu, W., Nie, X., Li, M., Wang, B., & Chen, W. (2022). A review of practical statistical methods used in epidemiological studies to estimate the health effects of multi-pollutant mixture. *Environmental Pollution*, 306, 119356. <https://doi.org/10.1016/j.envpol.2022.119356>
- [68] Zou, H., & Hastie, T. (2005). Addendum: Regularization and variable selection via the elastic net. *Journal of the Royal Statistical Society Series B: Statistical Methodology*, 67(5), 768. <https://doi.org/10.1111/j.1467-9868.2005.00527.x>

Supplementary material

Devido ao extensivo material suplementar (mais de 100 páginas de arquivo), o material ficará disponível no link abaixo.

Link: [Material Suplementar Capítulo 2](#)

CAPÍTULO 3:

Spatiotemporal dynamics of physical, chemical and biological water quality parameters in the Paraíba do Sul River Basin: A decade of data analysis

Pretensão de Submissão – **Anais da Academia Brasileira de Ciências**

Qualis-Capes (Área de Biodiversidade, 2021-2024): A3

Fator de Impacto (2022): 1.3

Status: Submetido

CAPÍTULO 3 - Spatiotemporal dynamics of physical, chemical and biological water quality parameters in the Paraíba do Sul River Basin: A decade of data analysis

Échily Sartori (*), Diego Lacerda, Cristiane dos Santos Vergilio, Luis Schiesari, Carlos Eduardo de Rezende

É. Sartori, D. Lacerda, C. E de Rezende

Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Avenida Alberto Lamago, 2000, Parque Califórnia, Campos dos Goytacazes, Rio de Janeiro 28013-602, Brazil.

É. Sartori

e-mail: echilysartori1@gmail.com; ORCID: <https://orcid.org/0000-0002-1214-5253>

D. Lacerda

e-mail: lacerdadesouza28@gmail.com; ORCID: <https://orcid.org/0000-0001-6237-6765>

C. E. de Rezende

e-mail: crezende@uenf.br; ORCID: <https://orcid.org/0000-0003-2804-8930>

C. S. Vergilio

Departamento de Biologia, Centro de Ciências Exatas Naturais e da Saúde, Universidade Federal do Espírito Santo – Campus Alegre, Alto Universitário, S/N, Alegre, Espírito Santo 29500-000, Brazil

e-mail: cristianesvergilio@gmail.com; ORCID: <https://orcid.org/0000-0003-1711-7320>

L. Schiesari

Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, Avenida Arlindo Bettio 1000, São Paulo, São Paulo 03828-000, Brazil

e-mail: lschiesa@usp.br; ORCID: <https://orcid.org/0000-0003-0814-591X>

* Corresponding author

Abstract

This study investigated the spatiotemporal dynamics of physical-chemical, and biological parameters in the Paraíba do Sul River (PSR) basin from 2012 to 2023. Data on 29 water parameters were analyzed from multiple monitoring agencies. Results indicated concerning trends, with variables such as dissolved Al, Fe, total P, and Mn exceeding thresholds set by Brazilian legislation. Regression analysis showed increases in contaminants like *E. coli*, chloride, dissolved Al and Fe, nitrate, P and Mn, alongside reduced dissolved oxygen and an increase in acidity at the main river course over time. Seasonal variations showed higher concentrations of pollutants like Al, Fe, total organic carbon, and microbiological indicator of fecal contamination (i.e. *E. coli*) during the rainy season. Spatial analyses revealed higher concentrations of these parameters in the upper PSR, associated with urban, industrial, and agricultural areas. A dilution of contaminants was observed along the downstream gradient, with the effect being more pronounced in areas less affected by anthropogenic inputs. In contrast, the highest contamination concentrations were recorded in the most heavily impacted, and regions that received waste from different accidents. Principal Component Analysis identified key variables associated with contamination, such as metals, organic matter, and fertilizers, highlighting the need for targeted interventions and improved waste management strategies in the upper and middle portion.

Key words: Anthropogenic pollution, Ecological impact, Environmental contamination, Metal, Microbiological indicator, Reservoir.

Introduction

Water is a natural resource essential for sustaining life on Earth. However, increasing population and industrial growth have significantly contributed to the generation of domestic and industrial effluents, which are often improperly discharged into river systems. In this context, regular monitoring of water quality is crucial, as it enables the identification of pollutants and contaminants that, when present at levels exceeding allowed thresholds, can compromise its chemical, physical, and biological integrity. These impacts represent a risk not only to aquatic ecosystems but also to human health (Silva et al., 2022; Wu et al., 2023; Rai et al., 2024).

The Paraíba do Sul River (PSR) drains one of the most developed and densely populated regions of Brazil, where intense industrial, agricultural, livestock, and mining activities influence surface water quality and biogeochemical dynamics (AGEVAP,

2006; Pfeiffer et al., 1986; Lacerda, 1996; Ovalle et al., 2013; Pacheco et al., 2017; Tonhá et al., 2021; Gomes et al., 2023). Dams for energy generation and water supply are distributed throughout the basin, and approximately 80% of sewage is discharged untreated into the river (AGEVAP, 2020). These combined stressors have led to a long history of contamination and ecosystem degradation in the PSR.

Since the 1980's the PSR has also experienced numerous disasters incidents involving spills of industries effluents, mining tailing and illegal gold mining, further highlighting the need for long-term water quality monitoring. Notable events include multiple spills into rivers on the left bank, such as the 1982 spills of Cd, Pb, and Zn by Companhia Paraibuna Metais into the Paraibuna River. Othe event include 1984 sulfuric acid spill into the Piabanha River, the 1986 Ascarel (PCB-derived compound) oil spill, and the 1989 methanol spill in Barra do Piraí, which disrupted water supply to municipalities. Additionally, algal blooms (2002, 2004, 2007), the 2003 rupture of a caustic soda reservoir by Cataguases de Papel into the Pomba River, as well as repeted bauxite sludge spills (2006, 2007), further compromised water quality, along with Endosulfan (2008) on the Pirapetinga River and Hg contamination from gold mining in 2009/2010 in the middle and lower PSR (O Globo, 2009; Ovalle et al., 2013; CBH-BPSI, 2014; Wanderley et al., 2014; Álvarez & Valpassos, 2023). These historical accidents, along with the ongoing environmental pressure, underscore the urgency of continuous monitoring to prevent further degradation of water quality, to prioritize regions and river reaches for restoration, and to ensure sustainable watershed management.

While numerous studies have made significant contributions in the assessment of water quality in the PSR basin through short-term analyses (Pfeiffer et al., 1986; Lacerda, 1997; Pacheco et al., 2017; Tonhá et al., 2021), fewer have investigated long-term trends. Among those who have conducted studies, many have provided important findings by focusing on a single variable (Gomes et al., 2023) or adopting a multivariable approach (Ovalle et al., 2013). Notably, robust time-series analyses have revealed the influence of ENSO events and drought conditions on base-level fluctuations of certain elements in the basin (Ovalle et al., 2013). However, these previous efforts were either restricted to specific parameters or limited spatial coverage. The novelty of our study lies in providing a basin-wide integration of multiple monitoring programs, enabling a comprehensive multivariable spatiotemporal

assessment across the entire PSR basin over a decade, thereby addressing a critical gap in water quality diagnostics.

Spatiotemporal analyses are critical for understanding how contaminants fluctuate due to seasonal patterns, anthropogenic pressures, and natural processes. This study leverages a 12-year dataset (2012-2023) from three monitoring agencies, a temporal coverage and multivariable approach for the PSR basin. Despite the recognized importance of these approaches worldwide, no study has yet performed a long-term, comprehensive, multiple variable assessment across the entire PSR basin that integrates both spatial gradients and decadal trends.

Numerous studies worldwide have reported concerning levels of environmental degradation and variability in physical, chemical, and biological parameters, often driven by land use, pollution sources, hydrological changes, and dam-induced alterations in flow regimes (Ouyang et al., 2005; Hatje et al., 2017; Vergilio et al., 2020; Vergilio et al., 2021; Gomes et al., 2023; Wu et al., 2023). These findings reinforce the urgent need for long-term, multivariable monitoring programs, particularly in complex systems such as the PSR basin, where short-term analyses fail to capture critical temporal and spatial patterns.

There are evident and ongoing concerns about the degradation of water quality in the PSR basin, yet critical questions remain regarding how water quality changes over time and across different regions. For instance: (1) which variables most frequently exceed the established regulatory limits in the current scenario (2023) and compared to the historical reference period (2012–2014); (2) how do these exceedances vary longitudinally along the river course; and (3) how do these variables fluctuate seasonally between wet and dry periods? Addressing these questions is essential to identify priority areas for intervention and support the development of effective water management strategies in the PSR basin.

Material e Methods

Study area

The Paraíba do Sul River (PSR) Basin (Fig. 1), located in southeastern Brazil, covers a drainage area of 62,074 km² across the states of São Paulo (22.7%), Rio de Janeiro (43.7%), and Minas Gerais (33.6%). It is a highly urbanized basin that shelters ~9.6 million people. Additionally, through inter-basin water transfers to the Guandu

River system, the PSR supplies water to the entire Rio de Janeiro Metropolitan Region, with ~ 13 million inhabitants. The river is formed by the confluence of the Paraíba and Paraitinga rivers in the state of São Paulo and extends for about 1,150 km until its mouth in northern Rio de Janeiro State (AGEVAP, 2020, 2022). The climate is strongly seasonal, with well-defined dry and wet seasons from April to September and October to March of the following year, respectively (Marques et al., 2017; Cotovicz et al., 2020; Serafim et al., 2023). The average flow rate is ~814 m³/s, with higher discharges during the wet season and lower discharges during the dry season (AGEVAP, 2006).

The PSR basin is subdivided into three sectors: (1) The Upper PSR, located in São Paulo state, serves as a high-tech industrial hub with aerospace, automotive and chemical industries, alongside well-balanced land use (15.6% forest cover - highest in the basin) and advanced infrastructure supporting its 57.6% pasture and 4.5% agricultural areas, it's also notable by hydroelectric power plants and reservoirs; (2) The Middle PSR, emerges as the dominant economic center with 434 metal industries, 1,016 food/drink plants, and 1,589 textile businesses, and suffers severe environmental degradation (70.7% pasture, just 9.2% forest cover). This sector also is known for hydroelectric power plants and reservoirs; and (3) The Lower PSR, focused on sugarcane agriculture and coastal industries, where 61.2% pasture and 13.2% forest cover reflect transitional pressures. The Middle and Lower PSR are the sectors in which the accidents reported above have occurred (AGEVAP, 2006; Freitas et al., 2007; AGEVAP, 2020; Gomes et al., 2022).

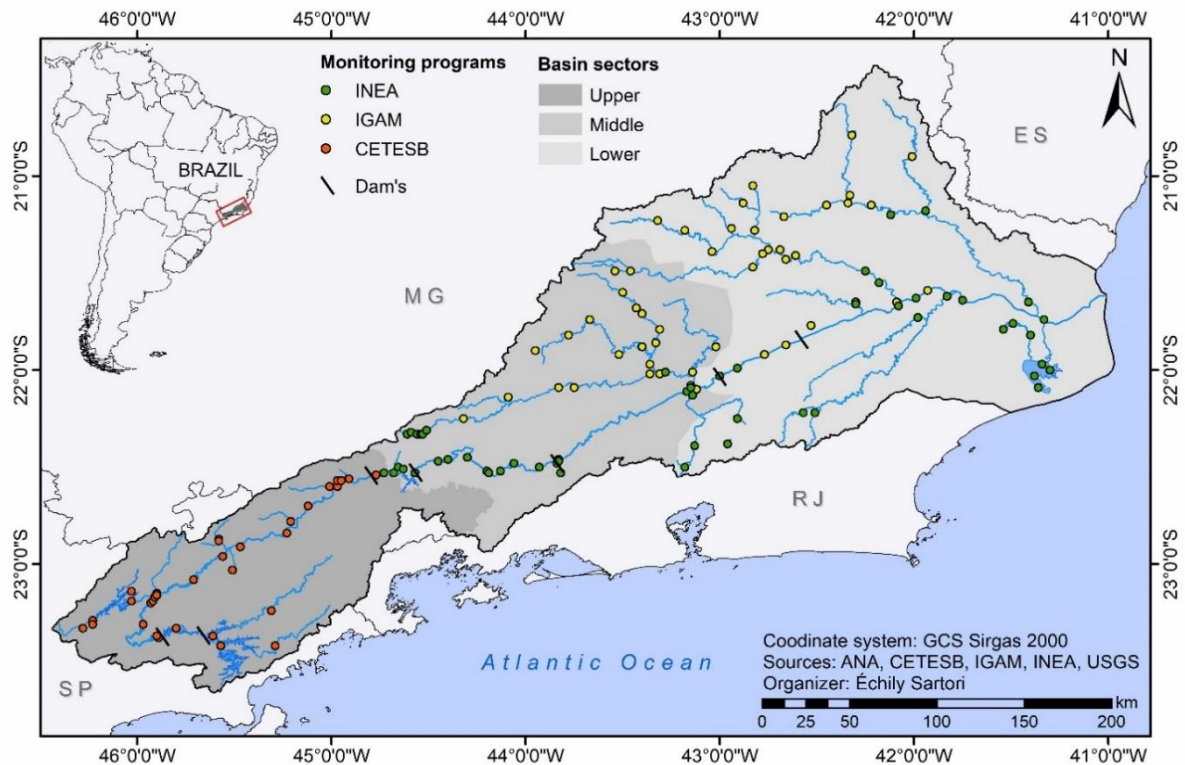


Figure 1 Water quality sampling stations in the Paraíba do Sul River Basin, Southeastern Brazil. The figure was prepared using the software ArcGIS (ArcMap 10.8 version 10.7.0.10450; <https://desktop.arcgis.com/en/>).

Hydrological monitoring: Programs and data selection

Water monitoring in the Upper PSR is conducted by the Companhia Ambiental do Estado de São Paulo (CETESB), while the Middle and Lower PSR sector are monitored by the Instituto Mineiro de Gestão das Águas (IGAM) in Minas Gerais and the Instituto Estadual do Ambiente (INEA) in Rio de Janeiro. CETESB and IGAM have established a pattern of quarterly sampling of chemical, physical, and biological variables across 86 Hydrological Units for Water Resources Management (HUWRM) in the basin. In contrast, INEA, which monitors 56 HUWRM, has not maintained a regular sampling schedule, and some variables are monitored sporadically, with intervals of several years between samples.

To support this study, the selection of data sets was based on the availability of online data provided by the three monitoring agencies. A 12-year time series (Jan 2012 to Dec 2023) was selected, including 29 variables deemed informative for assessing river water quality and simultaneously recorded by the three agencies. The parameters analyzed included total alkalinity; dissolved metals (Al, Cu, Fe); total metals (As, Cd, Cu, Cr, Fe, Pb, Mn, Hg, Ni, Zn); total organic carbon (TOC); total phenols; nutrients

(nitrate, nitrite, total phosphorus); physical-chemical parameters (pH, dissolved oxygen [DO], conductivity, total dissolved solids [TDS], total suspended solids [TSS], total solids [TS], turbidity, total chloride); and biological indicators (biochemical oxygen demand [BOD], *Escherichia coli*).

Data processing

Data from the three governmental agencies were compiled, and the geographical coordinates of the 141 HUWRM were extracted and plotted (Fig. 1, Table 1). For tributaries with multiple HUWRM, data were aggregated to simplify the graphical presentation. To facilitate the longitudinal visualization of water quality parameters along the main river course, distances were calculated as the linear distance from the confluence of each tributary with the Paraíba do Sul River towards the ocean. Full details on station grouping are provided in Supplementary Material 2. To establish a reference baseline of the river water conditions, data from 2012 to 2014 were aggregated, which allowed comparison with the current conditions (that is, conditions observed in 2023). We emphasize that this baseline does not represent pre-disturbance or minimally impacted conditions but rather reflects the earliest dataset with adequate spatial coverage and consistency for the entire PSR basin. The choice of these years (2012–2014) was based on the best consistency in INEA's sampling during this period, ensuring greater reliability for comparisons with data from the other two agencies. The dry and wet seasons were defined as April to September and October to March of the following year, respectively (Neves & Vilanova, 2021). This classification follows the well-marked tropical seasonality of the Paraíba do Sul River basin, where rainy months consistently correspond to high-flow conditions and dry months to low-flow periods, as reported in previous hydrological studies described in Study area.

Table 1. Geographical coordinates and characteristics of the watercourses in the PSR basin. Figure code represents the identification code in the figures, latitude and

longitude are given in decimal degrees, and distance is measured from the confluence of the Paraitinga and Paraibuna rivers towards the ocean.

Figure code	Lat	Long	Watercourse	Division	Bank	Distance (km)
1	-23.39191	-45.61413	Paraitinga/Paraibuna River	Upper	Confluence	0
2	-23.18381	-45.91823	Vidoca Stream	Upper	Right	123
3	-23.17380	-45.91412	Jaguari River	Upper	Left	125
4	-23.15369	-45.89669	Buquira River	Upper	Left	127
5	-22.92394	-45.54361	Piracuama River	Upper	Left	205
6	-22.92151	-45.52415	Una River	Upper	Right	209
7	-22.80832	-45.20742	Guaratingueta River	Upper	Left	253
8	-22.61182	-45.00052	Piquete River	Upper	Left	295
9	-22.57064	-44.95187	Pontilhão Stream	Upper	Left	302
10	-22.56669	-44.94928	Água Limpa Creek	Upper	Left	303
11	-22.56538	-44.91144	Jacu River	Upper	Left	307
12	-22.51106	-44.61839	Água Branca Stream	Middle	Left	342
13	-22.46490	-44.40695	Pirapetinga River	Middle	Left	376
14	-22.51440	-44.19569	Bananal River	Middle	Right	418
15	-22.46661	-43.82747	Piraí River	Middle	Right	468
16	-22.11349	-43.14517	Piabanha River	Middle	Right	564
17	-22.10637	-43.12981	Paraibuna River	Middle	Left	566
18	-21.80698	-42.51027	Angu River	Lower	Left	642
19	-21.71480	-42.26791	Pirapetinga River	Lower	Left	668
20	-21.64226	-42.06117	Pomba River	Lower	Left	692
21	-21.59725	-41.78885	Dois Rios River	Lower	Right	723
22	-21.71750	-41.36530	Muriaé River	Lower	Left	774
23	-21.75389	-41.32525	Ururá River	Lower	Right	779
24	-23.33470	-45.79530	Paraíba do Sul River	Upper		26
25	-23.37560	-45.88670	Paraíba do Sul River	Upper		43
26	-23.31330	-45.97220	Paraíba do Sul River	Upper		100
27	-23.19500	-45.93000	Paraíba do Sul River	Upper		121
28	-23.16250	-45.90310	Paraíba do Sul River	Upper		130
29	-23.07830	-45.71080	Paraíba do Sul River	Upper		165
30	-22.96420	-45.55890	Paraíba do Sul River	Upper		200

Figure code	Lat	Long	Watercourse	Division	Bank	Distance (km)
31	-22.91170	-45.47030	Paraíba do Sul River	Upper		215
32	-22.84440	-45.23440	Paraíba do Sul River	Upper		247
33	-22.70330	-45.11940	Paraíba do Sul River	Upper		272
34	-22.59580	-44.97110	Paraíba do Sul River	Upper		298
35	-22.54220	-44.77390	Paraíba do Sul River	Upper		326
36	-22.52878	-44.68068	Paraíba do Sul River	Middle		343
37	-22.52903	-44.56849	Paraíba do Sul River	Middle		353
38	-22.46690	-44.44793	Paraíba do Sul River	Middle		370
39	-22.45047	-44.30038	Paraíba do Sul River	Middle		404
40	-22.52577	-44.18995	Paraíba do Sul River	Middle		419
41	-22.51997	-44.13320	Paraíba do Sul River	Middle		426
42	-22.47845	-44.06302	Paraíba do Sul River	Middle		438
43	-22.49903	-43.93458	Paraíba do Sul River	Middle		455
44	-22.48223	-43.83918	Paraíba do Sul River	Middle		466
45	-22.46498	-43.82759	Paraíba do Sul River	Middle		469
46	-22.11000	-43.17000	Paraíba do Sul River	Middle		562
47	-22.10000	-43.12000	Paraíba do Sul River	Middle		569
48	-22.03472	-42.99639	Paraíba do Sul River	Lower		586
49	-21.99096	-42.90999	Paraíba do Sul River	Lower		596
50	-21.92000	-42.77000	Paraíba do Sul River	Lower		614
51	-21.87000	-42.66000	Paraíba do Sul River	Lower		625
52	-21.65000	-42.09000	Paraíba do Sul River	Lower		689
53	-21.62917	-41.98750	Paraíba do Sul River	Lower		700
54	-21.59000	-41.93000	Paraíba do Sul River	Lower		707
55	-21.64333	-41.74583	Paraíba do Sul River	Lower		732
56	-21.74444	-41.33056	Paraíba do Sul River	Lower		778

Data analysis

To provide an integrated overview of water quality across the entire PSR basin (main course and tributaries), the median values of the 5th, 50th, and 95th percentiles were calculated for both the baseline period (2012–2014) and the current dataset (2023) (Supplementary Material 2). An increase factor (IF=median 2023 value/ median

baseline value) was then determined. We adopted the median as the central tendency metric both due to the high occurrence of values below analytical quantification limits (QL) and to minimize the influence of outliers and enhance the stability of temporal comparisons. Values below QL were conservatively replaced with the corresponding QL value to maintain consistency across the dataset. All limits are available in the supplementary material 2. In parallel, to explore the spatial variability and exceedance patterns across individual monitoring stations, absolute values for each station/distance were used to calculate the magnitude and percentage of exceedances relative to both the baseline condition (2023 median / baseline median) and the national regulatory thresholds defined by CONAMA 357, class II (applicable to water bodies intended for human consumption supply after conventional treatment) (2023 observed data / CONAMA 357 limit). These calculated values were then used to generate diagnostic plots, which allow visualization of (i) the variables that most frequently exceed regulatory and baseline thresholds and (ii) the magnitude of these exceedances across the entire basin and within each of the defined river sections (Upper, Middle, and Lower PSR).

Linear regressions (lm, base package, R Core Team, 2024) were used to assess temporal trends in the response variables. The analysis was restricted to the PSR mainstem dataset, and all individual measurements were used to explicitly incorporate intra- and interannual variability. To evaluate seasonal differences, one-way ANOVA were applied (aov, base package, R Core Team, 2024). When necessary, data transformations were performed using the maximum likelihood method (boxcox, MASS package, Venables and Ripley, 2002) to meet assumptions of normality, linearity, homoscedasticity, and residual leverage. Regressions were conducted using univariate models, with each parameter evaluated independently. Model assumptions were validated using diagnostic plots (Altman & Krzywinski, 2016), and all analyses were conducted at a 95% confidence level. These models were intended to identify indicative temporal trends, without explicitly accounting for nested spatial structure or temporal autocorrelation.

For correlation and association between variables and basin sectors, Principal Component Analysis (PCA; pca, FactoMineR, R Core Team, 2024) was applied to the main dataset. Since PCA is sensitive to differences in measurement units, all variables were standardized using z-scores. All analysis was performed using R Core Team 2024.

Our analytical approach examines water quality through complementary but distinct dimensions, with each results section addressing specific research questions while collectively building a comprehensive assessment. Together, these sections provide non-redundant insights: (1) overall comparisons establish establishes degradation magnitudes against Brazilian legislation historical reference conditions, (2) reveal both long-term trends and seasonal variations, (2) spatial patterns identify contamination hotspots along longitudinal and cross-sectional gradients, and (4) multivariate statistics explain parameter covariation across basin sectors.

Results

Overall water quality status

Considering baseline values for the entire basin (main river + tributaries), some variables, including dissolved Al and Fe, total Cd, Pb, P, and Mn, exhibited 50th and/or 95th percentile values exceeding the limits established by the Brazilian legislation (Table 2) (CONAMA 357). Furthermore, under current conditions (2023 data), the parameters dissolved Al and Fe, total chloride, total Fe, Mn, Zn, TOC, conductivity, E. coli, nitrite, TS, and turbidity exhibit increased values compared to the baseline, while the medians of DO, pH, nitrate, TSS and TDS have decreased over the same period. It is important to note that red mark increases are primarily due to changes in QL. This must be considered when interpreting these changes.

Table 2. Global overview of decade-long temporal changes in water quality in the PSR Basin. 5th, 50th and 95th percentiles of 29 water quality parameters taken in the baseline period (2012–2014) and 2023 are contrasted (IF=median 2023 value/ median baseline value) and compared with national environmental standards (CONAMA 357/2005 Class 2 Water Bodies). Red values indicate parameters for which

quantification limits (QL) varied over time; therefore, for these parameters reported IFs do not reflect true concentration increases.

Parameters	Percentiles						CONAMA 357	IF
	2012 to 2014			2023				
	5%	Median (50%)	95%	5%	Median (50%)	95%		
Alkalinity	15.00	17.00	20.35	20.00	20.00	24.76		1.18
Al _{dis}	0.10	0.10	0.26	0.10	0.12	0.21	0.10	1.23
As _{total}	2.00	2.00	2.00	5.25	5.25	5.25	10.00	2.63
Cd _{total}	0.50	0.85	2.00	1.00	1.00	1.00	1.00	1.18
TOC	1.88	3.22	6.80	3.00	4.50	8.70		1.40
Pb _{total}	5.00	9.25	20.00	10.00	10.00	10.00	10.00	1.08
Chloride _{total}	3.28	4.09	8.51	3.84	5.48	8.22	250.00	1.34
Cu _{dis}	5.00	5.00	5.00	4.00	5.00	5.00	9.00	1.00
Cu _{total}	5.00	5.00	7.55	5.00	5.00	5.00		1.00
Conductivity	34.04	75.50	109.10	67.27	91.24	118.27		1.21
Cr _{total}	0.02	0.05	0.05	0.05	0.05	0.05	0.05	1.00
BOD	2.00	2.00	3.00	2.00	2.00	3.20	5.00	1.00
<i>E. coli</i>	206	1.95	14.9	635	4.4	19.03	600	2.26
Phenol _{total}	2.00	3.00	3.00	2.50	2.50	3.00	3.00	0.83
Fe _{dis}	0.08	0.20	0.55	0.21	0.42	0.63	0.30	2.10
Fe _{total}	0.40	0.98	2.92	0.72	1.08	1.44		1.10
P _{total}	0.02	0.06	0.14	0.02	0.06	0.15	0.10	1.00
Mn _{total}	0.03	0.06	0.11	0.05	0.10	0.18	0.10	1.74

Parameters	Percentiles						CONAMA 357	IF
	2012 to 2014			2023				
	5%	Median (50%)	95%	5%	Median (50%)	95%		
Hg _{total}	0.11	0.20	0.20	0.20	0.20	0.20	0.20	1.00
Ni _{total}	0.01	0.01	0.01	0.01	0.01	0.01	0.025	1.00
Nitrate	0.40	0.65	1.14	0.28	0.53	0.95	10.00	0.80
Nitrite	8.83	10.00	68.75	13.65	20.00	30.23	1000.00	2.00
DO	5.80	7.30	8.28	5.87	6.88	8.29	5.00	0.94
pH	6.70	7.20	8.00	6.51	6.98	7.35	6 and 9	0.97
TDS	42.10	56.00	84.50	50.00	53.50	85.70	500.00	0.96
TSS	2.00	10.50	57.30	2.00	9.50	59.70		0.90
TS	50.00	68.00	130.40	50.90	79.50	155.45		1.17
Turbidity	3.05	9.20	51.60	6.96	19.23	78.31	100.00	2.09
Zn _{total}	0.01	0.02	0.04	0.01	0.03	0.09	0.18	1.63

Figure 2 presents the magnitude of exceedances of the CONAMA 357 Class 2 limits, calculated as the ratio between the median concentration (50th percentile) and the regulatory threshold, for each water quality variable along the main course of the PSR (Figure 2a), as well as for the Upper (Figure 2b), Middle (Figure 2c), and Lower (Figure 2d) river sections. At the main course scale (Figure 2a), *E. coli*, dissolved Al (Al dis), and dissolved Fe (Fe dis) showed the highest exceedance ratios, with median values surpassing the regulatory limits. Turbidity, P, and total Mn also approached or slightly exceeded the limits at specific sites (Supplementary material 1 and 2). In the Upper PSR (Figure 2b), the exceedance magnitude was most pronounced for *E. coli*, dissolved Al, and dissolved Fe, indicating upriver contamination. Other parameters remained largely within acceptable limits. In the Middle PSR (Figure 2c), the number of parameters exceeding limits decreased compared to the Upper PSR; however,

localized violations for *E. coli* and dissolved Fe persisted with higher magnitudes. The Lower PSR (Figure 2d) showed a further reduction in the magnitude of exceedances, with only dissolved Fe and, to a lesser extent, *E. coli* maintaining values near or slightly above the regulatory threshold.

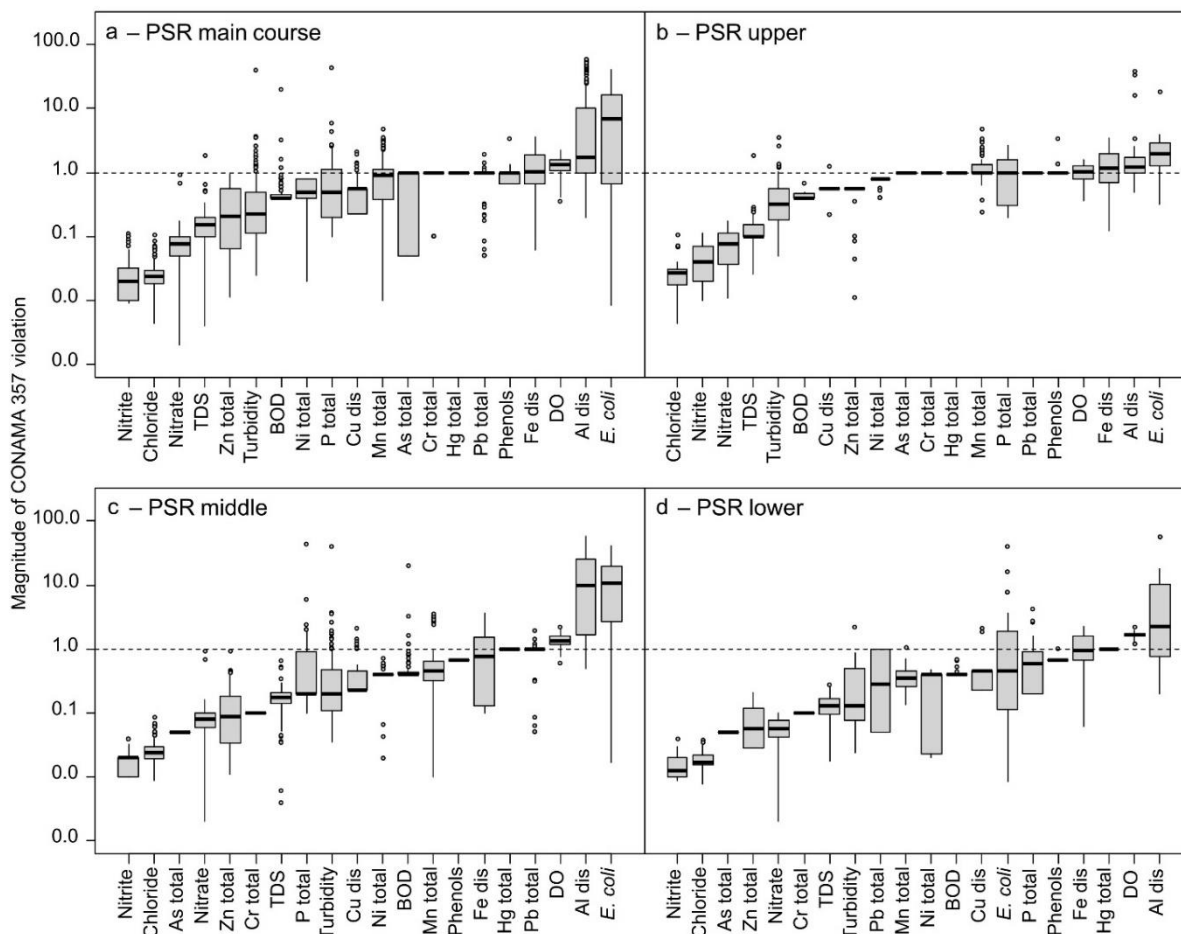


Figure 2 Magnitude of CONAMA 357 violations for environmental parameters along the main course of the Paraíba do Sul River (a) and its subdivisions (b–d) in 2023. Boxplots show the ratio between measured values and the national water-quality standard; ratios >1 indicate non-compliance. Note that (i) DO violations correspond to values *below* the standard (DO<1). QLs for total As, Hg, and Cd exceed the standards, preventing a meaningful compliance assessment. *E. coli* in the upper section was measured only at one HUWRM near the middle section (see Supplementary Material 2).

Overall, the figure illustrates a decreasing trend in the number of regulatory exceedances from upstream to downstream, suggesting the influence of natural attenuation or dilution processes along the river continuum. However, while violations became less frequent downstream, the magnitude of exceedances for key parameters

(e.g., *E. coli* and dissolved Al) peaked in the Middle PSR before declining again in the Lower PSR. This pattern indicates localized persistence of contamination despite broader improvements.

Although only a few variables exceeded the current regulatory limits, a larger number of variables showed increases when comparing current median values to baseline medians (Fig. 3). Across the entire river course (Fig. 3a), 17 variables presented median values above the baseline. The most prominent increases were observed for turbidity, dissolved Fe, and total Zn, with median concentrations approximately twice as high as the baseline values. In the Upper PSR (Fig. 3b), four variables showed pronounced increases, with median concentrations approximately two or more times above the baseline: nitrite, TSS, turbidity, and total Zn. In the Middle PSR (Fig. 3c), notable increases were detected for dissolved Al and Fe, and turbidity. The Lower PSR (Fig. 3d) exhibited the smallest deviations relative to the baseline, with substantial increases observed only for dissolved Fe and total P (P total).

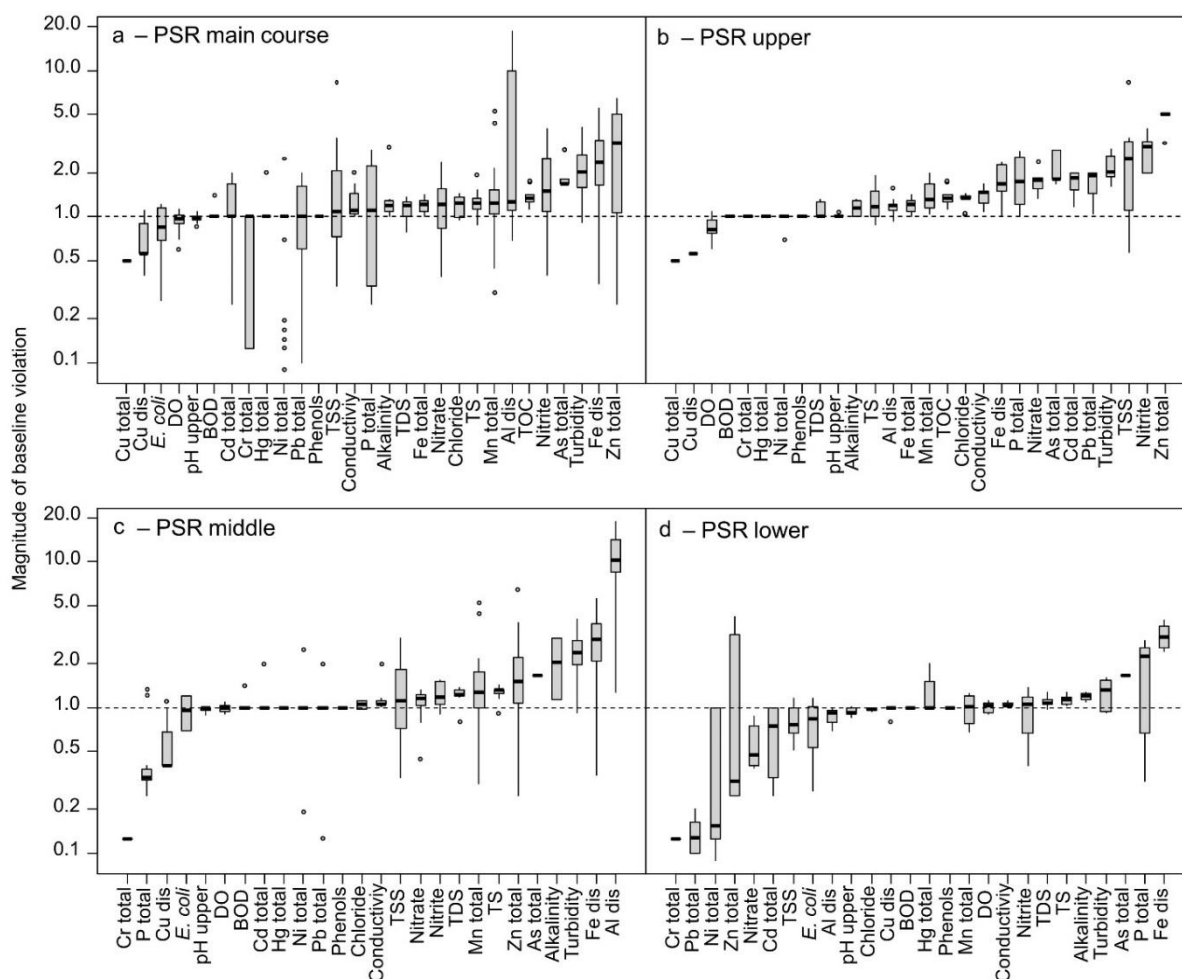


Figure 3 Magnitude of baseline violations for different environmental parameters along the principal course of the Paraíba do Sul River and its subdivisions. Boxplots represent the distributions of the ratios of recorded values; values above 1 indicate that the parameters exceeded the baseline established during the reference period (2012-2014), signaling a decrease in water quality over the last decade.

Overall, about half of the variables presented current median values near or above their respective baseline values (Supplementary Material 1; Figures/Pages 98–101 and Supplementary Material 2). The trend of upstream-to-downstream reduction seen for regulatory threshold violations was not as evident for baseline exceedances, indicating spatially heterogeneous temporal shifts in water quality across the PSR (Fig. 3).

Temporal trends: Interannual annual and seasonal variations

Several parameters exhibited concerning patterns of change over time. In the main river course, parameters such as DO (slope = -0.019 mg/L, $p < 0.03$) and pH (-0.016 u. pH; $p < 0.0001$) showed a significantly decreasing trend, while others such as dissolved Fe (Fig. 4a; slope 0.01 mg/L; $p < 0.0001$), TOC (slope 0.20 mg/L; $p < 0.0001$),

chloride (slope 0.01 mg/L; $p < 0.0001$), conductivity (slope 1.21 mg/L; $p < 0.0001$), BOD (slope 0.10 mg/L; $p < 0.0001$), and total P (Fig. 4b; slope 0.003 mg/L; $p < 0.001$) displays an increasing trend over the years (supplementary material 1, figures/pages 61 to 89). Furthermore, almost all physicochemical parameters analyzed in this study showed significant variations ($p < 0.05$), highlighting these trends in their behavior.

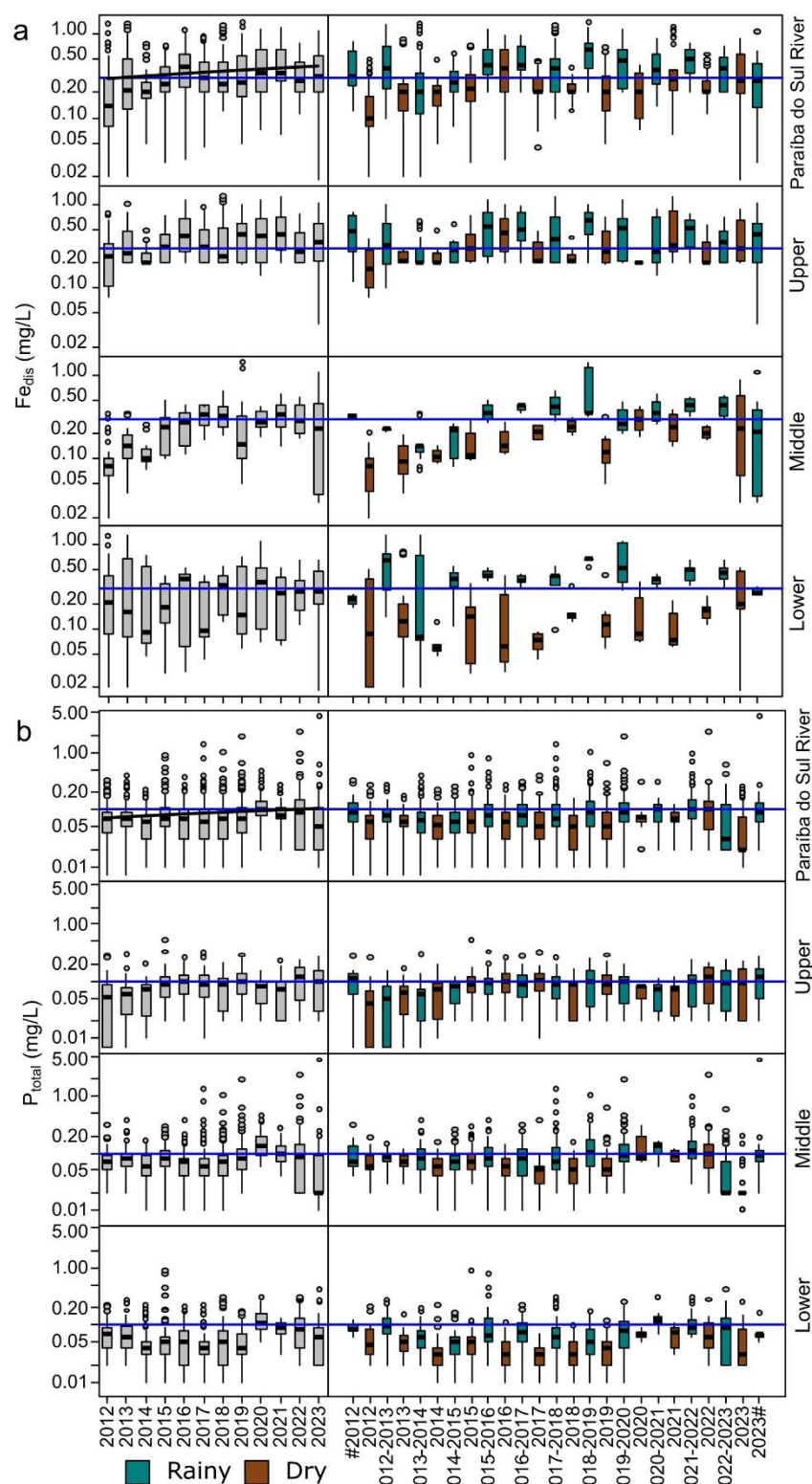


Figure 4 Temporal trends (left) and seasonal cycles (right) of dissolved Fe (a) and total phosphorus (b) from 2012 to 2023 in the main course of the Paraíba do Sul River. The horizontal blue line represents the threshold established by CONAMA 357 legislation. The black line on the Paraíba do Sul section is the fitted regression model.

Changes in seasonal concentration levels are also evident ($p < 0.05$), with higher values observed during periods of greater discharge (rainy season) for dissolved Al and Fe, chloride, TOC, *E. coli*, P, total Fe and Mn, all solids and turbidity. On the other hand, variables like DO, nitrate and conductivity show higher values on dry season, while alkalinity, DBO, nitrite and pH exhibit no differences among the seasons (supplementary material 1). However, the interpretation of annual trends for variables such as total As, Cd, Pb, Cu, Hg and Cr, as well as dissolved Cu, is hindered due to the variability in the QL over the years and among the agencies (supplementary material 2).

Finer scale spatial patterns

The selection of variables for present in the current study was based on their relevance in representing both inorganic and organic contamination levels. These variables were chosen to encompass a broad range of water quality aspects, including metals, organic matter, and nutrients, which are key indicators of environmental degradation in river systems. The inclusion of these parameters ensures a comprehensive understanding of the contamination dynamics in the PSR Basin, while also facilitating the identification of spatial and temporal trends that are crucial for assessing long-term changes and informing management interventions.

Variable trends along the main course of the river revealed distinct spatial patterns. The dams present along the river course were shown to alter the dynamics of certain parameters. Chloride (supplementary material 1, figures/pages 9) and conductivity (Fig. 5a) exhibited intense additions upon entering industrial and urban regions (around 100 km). Dissolved Fe (Fig. 5b), total P (Fig. 5c), total Mn, nitrite (Fig. 2d) and turbidity (supplementary material 1, figures/pages 20, 24 and 30, respectively) increased progressively downstream until reaching the dams (350 km), where a marked decline was observed in the middle and lower portions. Dissolved Al remained stable until the first dam in the middle course (around 325 km), with increased levels thereafter (supplementary material 1, figure/page 4). *E. coli* concentrations reduced immediately after the dam (350 km) and increased further along the middle section of the river (supplementary material 1, figure/page 15). Nitrate concentrations (Fig. 5d) increased consistently downstream, whereas nitrite levels decreased downstream the

same dam. Variables such as DO, pH, TDS, TSS, and TS showed slight variation along the river.

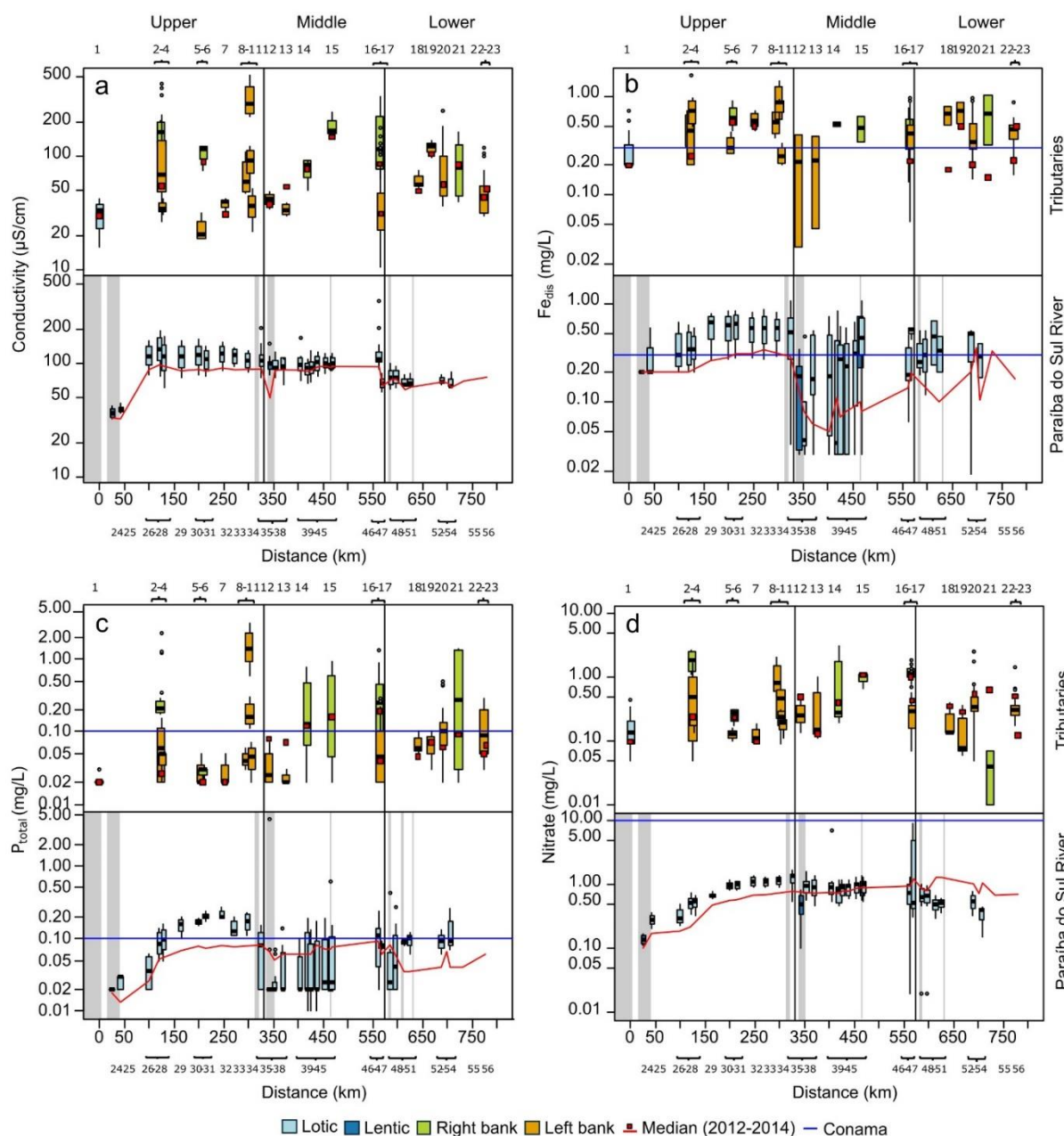


Figure 5 Variability in conductivity (a), dissolved Fe (b), phosphorus (c), and nitrate (d) along the Paraíba do Sul River and its tributaries in 2023. Sampling stations (1–56) are listed in Supplementary Materials 1 and 2; gray shading marks dams along the mainstem. For each parameter, the lower panel shows stations along the main course, while the upper panel shows tributary stations on the left (orange) and right (green) margins. Baseline conditions appear as a continuous red line in the lower panel, reflecting uninterrupted flow along the mainstem, and as separate red squares in the upper panel, corresponding to hydrologically independent tributaries.

Spatial analyses also revealed significant differences ($p < 0.05$) between the right and left banks of the PSR. Higher values of alkalinity, BOD, *E. coli*, total P, DO, and TS were observed on the left bank, whereas the right bank showed higher concentrations of dissolved Al, chloride, conductivity, nitrate, nitrite, pH, TDS and turbidity (Fig. 5 and supplementary material 1 and 2). In contrast, no significant spatial differences were observed for TOC, dissolved Fe and Mn, TSS, and total Zn. This spatial pattern indicates a marked division between the riverbanks, with the left bank predominantly influenced by variables related to organic load and biological activity, while the right bank exhibits elevated concentrations of physicochemical (conductivity, pH and turbidity) parameters typically associated with diffuse pollution sources or urban-industrial-land use inputs. For other variables, the interpretation was limited due to differences in QL adopted by IGAM and INEA.

Associations between variables and PSR basin sections

PCA was applied in the main river data to identify groups of variables with similar behavior and shared environmental origins (Fig. 6). Three principal components were retained for interpretation, explaining approximately 40% and 50% of the total variance in the baseline and current datasets, respectively. The unexplained variance reflects additional environmental drivers not fully aligned with the major gradients identified. In the baseline dataset (Fig. 6a), the first principal component (PC1; 17%) represents a metal–redox gradient, with strong positive loadings of Ni, Zn, and Mn, together with contributions from dissolved Fe, nitrite, and BOD, and opposed by marked negative loadings of DO and pH. These patterns indicate conditions in which reduced or moderately acidic environments facilitate the mobilization of redox-sensitive metals, reflecting the influence of anthropogenic inputs, sediment–water interactions, and oxygen depletion. The second component (PC2; 12%) is structured by dissolved Al and Fe, P, turbidity, and nitrate, capturing an enrichment gradient associated with suspended sediment inputs, nutrient availability, and runoff-derived materials. The third component (PC3; 10%) delineates an ionic and geochemical gradient dominated by chloride and conductivity, supplemented by nitrate and dissolved Al, indicating variation in ionic strength and mineralization.

In the current dataset (Fig. 6b), PC1 (19%) also reflects a metal–redox gradient, with positive contributions from Ni, Zn, Pb, Mn, dissolved Fe, and nitrite, contrasted

with a strong negative loading of DO. PC2 (17%) captures an organic–particulate enrichment gradient defined by BOD, P, and turbidity, reflecting shifts in organic loading, suspended solids, and eutrophication-related inputs. PC3 (11%) again represents an ionic–geochemical gradient, characterized by chloride, conductivity, nitrate, and dissolved Al.

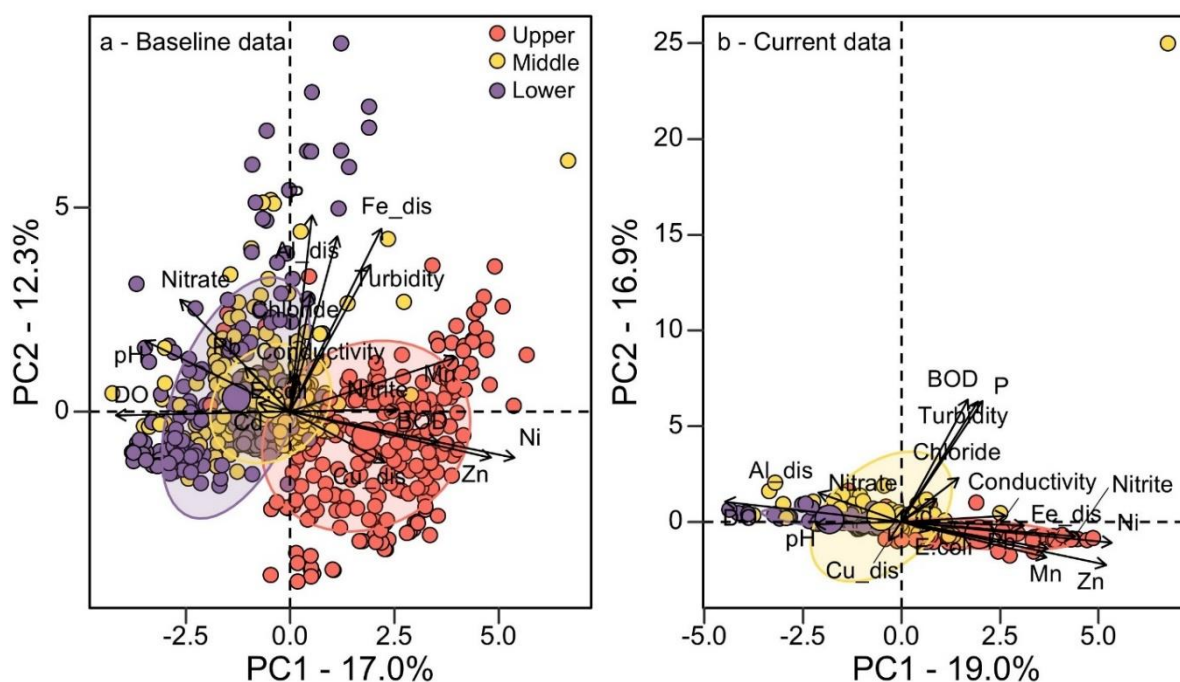


Figure 6 Principal Component Analysis (PCA) of water quality variables in the PSR basin, including metal concentrations, physicochemical parameters, and biological indicators. Panels show the baseline period (a) and 2023 data (b). Ellipses represent 68% confidence intervals for each basin sector.

Moreover, a comparison of the ellipse structures between the baseline and current datasets reveals a clear temporal shift in geochemical organization across the basin. In the baseline dataset (Fig. 6a), the ellipses are broader and more dispersed, with only partial overlap among basin sectors, indicating higher intra- and inter-group variability and more heterogeneous geochemical conditions. In contrast, the ellipses in the current dataset (Fig. 6b) are noticeably more compact and show stronger overlap, reflecting reduced internal variability and a convergence of geochemical signatures among sectors. This increased clustering suggests that the PSR basin has become more homogeneous over time, likely due to the cumulative influence of widespread and diffuse anthropogenic inputs that reduce spatial distinctions.

Discussion

The Paraíba do Sul River basin exhibits a complex water quality profile, with observed levels of various chemical, physical, and biological variables showing deviations from Brazilian legislation (CONAMA 357) and differing from baseline levels recorded a decade prior. This study investigates these patterns, providing an overview of the current state of water quality across the basin, focusing on the characteristics of these variables and their spatial and temporal distributions.

Recent studies using geoprocessing (Andrade et al., 2019; Paiva et al., 2020) and stable carbon isotopes (Serafim et al., 2023) have demonstrated the reduction and replacement of natural vegetation (Atlantic Forest) by urban, industrial, and agricultural areas throughout the years. These changes are reflected in the increase in sediment metals concentrations between 2 to 15 times in 10 years (Pfeiffer et al., 1986; Filho et al., 2002), and in the increase in other water variables like dissolved organic carbon and variables related to nitrogen and phosphorus (Ovalle et al., 2013). Although, in many cases, values are not observed outside the limits established by Brazilian environmental legislation, the magnitude of violations to our environmental baseline indicates a clear deterioration in the levels of variables that can be associated with sewage discharge, fertilizers, and industrial effluents. This is particularly evident in the Upper PSR sector (Fig. 3b), which concentrates on a high number of metallurgical, chemical, and other industrial hubs.

These results reflect the growing pressures caused by urbanization and human activities in the basin, such as industrialization, hydroelectric power plants, agriculture, livestock, and the inadequate treatment of sewage. Additionally, the basin has experienced significant environmental accidents, including chemical spills and industrial effluents. Between 2010 and 2022, the population within the basin grew by nearly 500 thousand inhabitants, with demographic density increasing from 126 to 147 inhabitants/km² (IBGE, 2010, 2022). This rapid urban and industrial expansion, coupled with intensified agricultural practices, has likely exacerbated the degradation of water quality and increased nutrient and contaminant discharges into the aquatic system.

The cumulative effect of these activities is reflected in the annual increase of both organic and inorganic variables, as well as the decrease in dissolved oxygen (DO) and pH levels, as observed through regression model slopes. The PSR basin reflects

centuries of anthropogenic transformation. Since the mid-20th century, particularly from the 1980s onward, the PSR basin has undergone complete anthropization, with over 80% of its area converted to urban, industrial, and agricultural uses (Pinto et al., 2023). This rapid transformation, marked by metropolitan expansion, the growth of industrial hubs, and agricultural intensification (especially sugarcane in the Lower PSR, Serafim et al., 2023) - has led to persistent inappropriate effluent discharges (Molisani et al., 1999; Tonhá et al., 2021; Vieira et al., 2022). Improper discharge of these effluents into the main river course and its tributaries, combined with intensive agrochemical and fertilizer use in agricultural areas (Pacheco et al., 2017), has raised contaminant levels in the basin. Brazil is responsible for about 8% of global fertilizer consumption, making it one of the four largest consumers (SAE, 2020). Phosphorus and nitrogen are the second and third most applied nutrients in Brazilian agriculture (MAP, 2022), significantly contributing to the increase in nutrient contamination in water bodies, thus promoting eutrophication and algal blooms.

Unlike observations that report lower concentrations of metals in water samples during rainy periods due to dilution effects for other river systems (Li & Zhang, 2010; Gogoi et al., 2020) and the PSR (Gomes et al., 2022), our results indicated higher concentrations of dissolved Al, total/dissolved Fe, TOC, P, turbidity, and *E. coli* during this period. This increase can be explained by the presence of accumulation areas that release these contaminants from sediments upon remobilization (Sartori et al., 2023), surface runoff (Agren et al., 2008; Rattan et al., 2017), and high temperatures (Freeman et al., 2009) typical of this time of year. On the other hand, the increase in DO levels during the dry season is associated with higher chlorophyll-a (Ovalle et al., 2013), likely resulting from the elevated P levels observed in the preceding months. The increase in organic variables such as P is closely related to water supply issues and disruptions in water availability for the population, due to its link with algal blooms. Therefore, constant monitoring of river waters is crucial to understanding and mitigating these impacts.

Spatial analyses revealed increasing concentrations of dissolved Fe, total P, Mn, nitrate, nitrite, and turbidity along the Upper PSR. Downstream, these elements undergo geochemical processes (dilution, flocculation) that reduce their concentrations. These patterns demonstrate how dams can significantly modify element dynamics along the river continuum. Pacheco et al. (2017) observed a similar trend in the Jacareí and São José dos Campos regions (~50 km), associating the

altered levels of parameters such as conductivity with the high number of local industries. Industrial effluents, rich in organic and inorganic contaminants, when improperly discharged, contribute to water quality degradation, negatively affecting both local biota and human health (Singh & Chandra, 2019; Okoyomon et al., 2021). However, the decrease in concentration observed near the 350 km mark is related to the Funil dam, which retains and reduces water flow, creating conditions for the adsorption and flocculation of certain contaminants onto suspended particles, which subsequently settle on the riverbed, reducing the bioavailability of some contaminants.

Although industrialization and urbanization have caused widespread contamination, the presence of reservoirs along the river further complicates the situation, influencing not only water quality but also the geochemistry of metals and nutrients (Hatje et al., 2017; Vergilio et al., 2020, 2021; Tomczyk et al., 2021). Additionally, Maavara et al. (2020) showed differences in the N and P cycles, highlighting changes in sedimentation, assimilation, and denitrification rates. These alterations can directly affect primary productivity both locally and downstream, as changes in nutrient fluxes may lead to eutrophication and algal blooms, compromising water supply, as observed in July 2024 in Campos dos Goytacazes (G1, 2024). The accumulation of pollutants in sediments within reservoirs may not only lead to periodic releases of contaminants into the water column but also create hotspots of metal toxicity, potentially affecting aquatic biota even long after the initial contamination events.

Despite the effects of reservoirs along the PSR, the levels of dissolved Fe, P, nitrate, turbidity and total Zn begin to rise again near the 460 km and 700 km marks, regions characterized by a high number of industries and agricultural areas, particularly in the Resende and Volta Redonda region (~460 km) and Campos dos Goytacazes (~700 km). Indeed, Filho et al. (2002) showed contamination levels of metals in the sediment in this region, while Andrade et al. (2019) documented changes in land use.

Although the tributaries on the left bank generally present higher concentrations of variables related to organic load and microbial activity, it is important to note that this region is more urbanized and consequently influenced by domestic effluent discharge. In addition, these sites are monitored by IGAM, which uses lower quantification limits and provides more consistent long-term data compared to INEA, potentially enhancing detection sensitivity. The presence of several reservoirs on the left bank (Gomes et al.,

2023) may also contribute to the retention of inorganic contaminants from industrial sources and past environmental incidents.

Therefore, continuous and long-term monitoring is essential for accurately assessing the quality of a river basin. Analyzing temporal trends allows for the identification of environmental degradation patterns and links them directly to anthropogenic activities. Furthermore, long-term monitoring is critical for the development of more effective public policies and management strategies that minimize damage to the aquatic ecosystem and ensure the sustainability of water resources. Standardizing monitoring methods across agencies will further enhance the reliability of data, providing a solid foundation for informed decision-making and effective environmental management.

Nonetheless, we acknowledge that differences in QL and sampling frequencies among CETESB, IGAM, and INEA introduce uncertainties. Although our conservative substitutions and use of medians (describe in the data analysis) reduce these biases, they cannot fully eliminate them. By explicitly flagging variables with changing QL (Table 2), we ensure cautious interpretation, while the consistency of observed trends across agencies supports the robustness of our findings despite these methodological constraints.

Conclusion

This study highlights the urgent need for long-term water quality monitoring in the Paraíba do Sul River (PSR), given the inconsistencies across agencies. Spatial and temporal analyses revealed clear deterioration trends, with multiple chemical, physical, and biological parameters exceeding CONAMA 357 standards and historical baselines. The upper PSR, under strong industrial and urban pressures, and the middle PSR, marked by recurrent environmental accidents, showed the most severe degradation.

Population growth, industrial expansion, and intensive fertilizer use have compounded contamination, increasing nutrient loads and chemical discharges. These pressures, combined with seasonal runoff and sediment resuspension, explain higher rainy-season concentrations of dissolved Al, Fe, TOC, P, turbidity, and *E. coli*, alongside declining DO and rising acidity. Spatial analyses identified hotspots between 50–350 km, strongly influenced by industrial hubs and locally mitigated by dams such

as Funil. Principal Component Analysis (PCA) revealed underlying gradients shaping water quality dynamics, including a metal–redox component, an enrichment gradient associated with suspended sediments, nutrients, and organic particulates, and an ionic/geochemical gradient. These results highlight how industrial discharges, sewage effluents, and agricultural runoff collectively drive contamination patterns across the basin.

Despite methodological challenges, including differing quantification limits (QL) and sampling frequencies among CETESB, IGAM, and INEA, conservative substitutions and the use of medians ensured robust comparisons. Variables with changing QL were flagged (Table 2), and consistent trends across agencies support the reliability of findings. Importantly, the baseline period (2012–2014) was chosen for data availability and consistency, not as a pristine condition. Thus, deterioration reflects intensification of contamination over the last decade, rather than changes from an undisturbed state.

To enable precise pollution source attribution, future monitoring must integrate high-resolution land-use data with critical dimensions, such as crop-specific cover, urban gradients, and georeferenced industrial discharge points. This approach will allow accurate emission factor calculations, and urban point sources.

Statements and Declarations

Conflict of Interest

The authors declare that they have no competing interests that are relevant to the content of this article.

Ethical Approval

This study did not involve experiments with human participants or vertebrate animals. All data analyzed were obtained from publicly available environmental monitoring programs conducted by Brazilian environmental agencies. Therefore, ethical approval was not required.

Informed Consent

Not applicable.

Clinical Trial Registration

Not applicable.

Data Availability

All data supporting the findings of this study are available within the article and its Supplementary Information. The environmental monitoring datasets used in the analyses are publicly available through the official data portals of Brazilian environmental agencies.

Funding

This research was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; grant no. 88887.824229/2023-00) and by CAPES in cooperation with the German Academic Exchange Service (DAAD; grant no. 88887.989816/2024-00), awarded to Échily Sartori. Additional support was provided to Diego Lacerda by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; grant no. E-26/200.316/2020), and to Cristiane dos Santos Vergilio by the Fundação de Amparo à Pesquisa do Espírito Santo (FAPES). Support to Luis Schiesari was provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; grant no. 310333/2022-9). Carlos Eduardo de Rezende received support from FAPERJ (grants E-26/206.117/2022 and E-26/203.950/2024) and from CNPq (grants 310.260/2023-0 and 409.378/2022-4). This study was also partially funded by CAPES – Finance Code 001.

Acknowledgments

The authors are grateful to colleagues and collaborators who contributed to the development of this work.

Author Contributions

Échily Sartori: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – Original Draft, Writing – Review & Editing, Visualization.

Diego Lacerda: Conceptualization, Methodology, Data curation, Writing – Review & Editing, Visualization.

Cristiane dos Santos Vergilio: Writing – Review & Editing, Supervision, Funding acquisition.

Luis Schiesari: Conceptualization, Writing – Review & Editing.

Carlos Eduardo de Rezende: Writing – Review & Editing, Supervision, Funding acquisition.

References

- AGEVAP - Associação Pro-Gestão das Águas da Bacia Hidrográfica do Rio Paraíba do Sul. (2020). Relatório sobre cenário ambiental 2020. 171p. <https://www.ceivap.org.br/>
- AGEVAP - Associação Pro-Gestão das Águas da Bacia Hidrográfica do Rio Paraíba do Sul. (2006). Plano de Recursos Hídricos da Bacia do Rio Paraíba do Sul – Diagnóstico dos Recursos Hídricos, Relatório Parcial. 232p. <https://www.ceivap.org.br/>
- AGEVAP – Associação Pro-Gestão das Águas da Bacia Hidrográfica do Rio Paraíba do Sul. (2022). Relatório sobre cenário ambiental 2022. 32p. <https://cbhmedioparaiba.org.br/relatorio-ambiental.php>
- Agren, A., Jansson, M., Ivarsson, H., Bishop, K., & Seibert, J. (2008). Seasonal and runoff-related changes in total organic carbon concentrations in the River Öre, Northern Sweden. *Aquatic Sciences*, 70, 21-29.
- Álvarez, S., & Valpassos, C. A. M. (Eds.). (2023). Alguns olhares do Sul: Antropologia, etnografia, e análise de conflitos e crises no século XXI. *EDUENF*, 95-103.
- Andrade, M. P. D., & Ribeiro, C. B. D. M. (2020). Impacts of land use and cover change on Paraíba do Sul watershed streamflow using the SWAT model. *Brazilian Journal of Water Resources*. Rbrh, 25, e12.
- Comitê de Bacia Hidrográfica do Baixo Paraíba do Sul e Itabapoana (CBH-BPSI). (2014). Boletim das Águas da Bacia Hidrográfica do Baixo Paraíba do Sul e Itabapoana: Nota técnica CBH BPSI: Decisão do Comitê do Baixo Paraíba do Sul e Itabapoana acerca da redução de vazões de chegada na transposição de Santa Cecília. Comitê de Bacia Hidrográfica do Baixo Paraíba do Sul e Itabapoana, Câmara Técnica de Recursos Hídricos e Estruturas Hidráulicas. Campos dos Goytacazes: CBH BPSI.

- Cotovicz, L. C., Vidal, L. O., de Rezende, C. E., Bernardes, M. C., Knoppers, B. A., Sobrinho, R. L., Cardoso, R. P., Muniz, M., dos Anjos, R. M., Biehler, A., & Abril, G., 2020. Carbon dioxide sources and sinks in the delta of the Paraíba do Sul River (Southeastern Brazil) modulated by carbonate thermodynamics, gas exchange and ecosystem metabolism during estuarine mixin. *Marine Chemistry*, 226. <https://doi.org/10.1016/j.marchem.2020.103869>
- Filho, S. R., Ribeiro Júnior, M. G., Alcover Neto, A., Silva, M. D. M., Zimbres, E., Yallouz, A. V., & Araújo, J. F. M. D. (2002). Evolução temporal da poluição por metais pesados em sedimentos do rio Paraíba do Sul, estado do Rio de Janeiro. (Série Tecnologia Mineral, 26). CETEM/MCT.
- Freeman, J. T., Anderson, D. J., & Sexton, D. J. (2009). Seasonal peaks in *Escherichia coli* infections: possible explanations and implications. *Clinical Microbiology and Infection*, 15(10), 951-953.
- Freitas, C. M. de, Schütz, G. E., & Oliveira, S. G. de. (2007). Environmental sustainability and human well-being indicators from the ecosystem perspective in the Middle Paraíba Region, Rio de Janeiro State, Brazil. *Cadernos de Saúde Pública*, 23(suppl 4), S513–S528. <https://doi.org/10.1590/s0102-311x2007001600012>
- G1. (2024). Moradores de Campos relatam água com cor amarelada, mau cheiro e gosto ruim. G1. <https://g1.globo.com/rj/norte-fluminense/noticia/2024/07/25/moradores-de-campos-relatam-agua-com-cor-amarelada-mau-cheiro-e-gosto-ruim.ghtml>
- Gignac, G. E., & Szodorai, E. T. (2016). Effect size guidelines for individual differences researchers. *Personality and individual differences*, 102, 74-78.
- Gogoi, A., Taki, K., & Kumar, M. (2020). Seasonal dynamics of metal phase distributions in the perennial tropical (Brahmaputra) river: Environmental fate and transport perspective. *Environmental research*, 183, 109265.
- Gomes, P. R., Pestana, I. A., de Almeida, M. G., & de Rezende, C. E. (2023). The Paraíba do Sul River Basin and its coastal area as a study model of the mercury cycle: A meta-analytical review of three decades of research. *Journal of Hazardous Materials*, 460, 132442.

- Gomes, P. R., Pestana, I. A., Viana, P. P., de Almeida, M. G., de Rezende, C. E., & de Souza, C. M. M. (2022). Effects of dams on As and Hg concentrations in three southeastern Brazil fluvial systems: Ocean inputs, sources and seasonal dynamics among environmental compartments. *Science of The Total Environment*, 849, 157865.
- Hatje, V., Pedreira, R. M., de Rezende, C. E., Schettini, C. A. F., de Souza, G. C., Marin, D. C., & Hackspacher, P. C. (2017). The environmental impacts of one of the largest tailing dam failures worldwide. *Scientific reports*, 7(1), 10706.
- IBGE. (2010). *Cidades e Estados do Brasil*. <https://cidades.ibge.gov.br>
- IBGE. (2022). *Cidades e Estados do Brasil*. <https://cidades.ibge.gov.br>
- Jackson A, & Parnell A (2023). SIBER: Stable Isotope Bayesian Ellipses in R. R package version 2.1.9, <<https://CRAN.R-project.org/package=SIBER>>
- Kaiser, H. F. (1960). The application of electronic computers to factor analysis. *Educational and psychological measurement*, 20(1), 141-151.
- Lacerda, L. D. D. (1997). Contaminação por mercúrio no Brasil: fontes industriais vs garimpo de ouro. *Química nova*, 20, 196-199.
- Li Siyue, L. S., & Zhang QuanFa, Z. Q. (2010). Risk assessment and seasonal variations of dissolved trace elements and heavy metals in the Upper Han River, China. *Journal of Hazardous Materials*.
- Maavara, T., Chen, Q., Van Meter, K., Brown, L. E., Zhang, J., Ni, J., & Zarfl, C. (2020). River dam impacts on biogeochemical cycling. *Nature Reviews Earth & Environment*, 1(2), 103-116.
- MAP (Ministério da Agricultura e Pecuária). (2022). *Plano Nacional de Fertilizantes, Estatísticas do Setor*. Governo Federal. <https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-agricolas/fertilizantes/plano-nacional-de-fertilizantes/estatisticas-do-setor>
- Marques, J. S. J., Dittmar, T., Niggemann, J., Almeida, M. G., Gomez-Saez, G. V., & Rezende, C. E., 2017. Dissolved Black Carbon in the Headwaters-to-Ocean Continuum of Paraíba Do Sul River, Brazil. *Frontiers in Earth Science*, 5. <https://doi.org/10.3389/feart.2017.00011>

- Molisani, M. M., Salomão, M. S. B., Ovalle, A. R. C., Rezende, C. E., Lacerda, L. D., & Carvalho, C. E. V. (1999). Heavy metals in sediments of the Lower Paraíba do Sul River and Estuary, R.J., Brazil. *Bulletin of Environmental Contamination and Toxicology*, 63(6), 682–690.
- Neves, A. D. O., & Vilanova, M. R. N. (2021). Caracterização da seca histórica da década de 2010 na Bacia do Rio Paraíba do Sul, Estado de São Paulo, Brasil. *Engenharia Sanitaria e Ambiental*, 26(2), 339-349.
- O Globo. (2009). Exploração de ouro no Paraíba do Sul vira caso de polícia. O Globo. <https://oglobo.globo.com/economia/exploracao-de-ouro-no-paraiba-do-sul-vira-caso-de-policia-3126890>
- Okoyomon, O. O., Kadir, H. A., Zango, Z. U., Saidu, U., & Nura, S. A. (2021). Physicochemical composition and heavy metal determination of selected industrial effluents of Ibadan City, Nigeria. *Open Journal of Environmental Research* (ISSN: 2734-2085), 2(2), 58-66.
- Ovalle, A. R. C., Silva, C. F., Rezende, C. D., Gatts, C. E. N., Suzuki, M. S., & Figueiredo, R. O. (2013). Long-term trends in hydrochemistry in the Paraíba do Sul River, southeastern Brazil. *Journal of hydrology*, 481, 191-203.
- Pacheco, F. S., Miranda, M., Pezzi, L. P., Assireu, A., Marinho, M. M., Malafaia, M., ... & Ometto, J. P. (2017). Water quality longitudinal profile of the Paraíba do Sul River, Brazil during an extreme drought event. *Limnology and Oceanography*, 62(S1), S131-S146.
- Paiva, A. C. E., Nascimento, N., Rodriguez, D. A., Tomasella, J., Carriello, F., & Rezende, F. S. (2020). Urban expansion and its impact on water security: The case of the Paraíba do Sul River Basin, São Paulo, Brazil. *Science of the Total Environment*, 720, 137509.
- Pfeiffer, W. C., Fiszman, M., Malm, O., & Azcue, J. M. (1986). Heavy metal pollution in the Paraíba do Sul River, Brazil. *Science of the Total Environment*, 58(1-2), 73-79.
- Pinto, L. F. G., Ferreira, J., Berenguer, E., & Rosa, M. (2023). Governance lessons from the Atlantic Forest to the conservation of the Amazon. *Perspectives in Ecology and Conservation*, 21(1), 1–5. <https://doi.org/10.1016/J.PECON.2022.10.004>

- Rai, K., Misra, A. K., Ranjan, R. K., Wanjari, N., Rajak, R., Yadav, S. K., ... & Khan, M. A. (2024). Assessment of Heavy Metal and E. coli Contamination in Water Sources of the East and South Districts, Sikkim Himalaya, India. *Water Conservation Science and Engineering*, 9(1), 22.
- Rattan, K. J., Corriveau, J. C., Brua, R. B., Culp, J. M., Yates, A. G., & Chambers, P. A. (2017). Quantifying seasonal variation in total phosphorus and nitrogen from prairie streams in the Red River Basin, Manitoba Canada. *Science of the Total Environment*, 575, 649-659.
- SAE (Secretaria Especial de Assuntos Estratégicos). (2020). Produção Nacional de Fertilizantes. Estudo Estratégico. Governo Federal https://www.gov.br/planalto/pt-br/assuntos/assuntos-estrategicos/documentos/estudos-estrategicos/sae_publicacao_fertilizantes_v10.pdf
- Sartori, É., dos Santos Vergilio, C., Gatts, P. V., de Oliveira, B. C. V., de Almeida, M. G., Thompson, F., & de Rezende, C. E. (2023). Trace metal concentration along the Brazilian coast: An assessment of the influence of the Doce River plume. *Marine Pollution Bulletin*, 188, 114640.
- Serafim, T. S. G., de Almeida, M. G., Thouzeau, G., Michaud, E., Niggemann, J., Dittmar, T., & de Rezende, C. E. (2023). Land-use changes in Amazon and Atlantic rainforests modify organic matter and black carbon compositions transported from land to the coastal ocean. *Science of the Total Environment*, 878, 162917.
- Silva, G. M. E., Campos, D. F., Brasil, J. A. T., Tremblay, M., Mendiando, E. M., & Ghiglieno, F. (2022). Advances in technological research for online and in situ water quality monitoring—A review. *Sustainability*, 14(9), 5059.
- Singh, A. K., & Chandra, R. (2019). Pollutants released from the pulp paper industry: Aquatic toxicity and their health hazards. *Aquatic toxicology*, 211, 202-216.
- Stekhoven, D. J., (2022). missForest: Nonparametric Missing Value Imputation using Random Forest. R package version 1.5.
- Tonhá, M. S., Araújo, D. F., Araújo, R., Cunha, B. C., Machado, W., Portela, J. F., ... & Garnier, J. (2021). Trace metal dynamics in an industrialized Brazilian river: A

- combined application of Zn isotopes, geochemical partitioning, and multivariate statistics. *Journal of Environmental Sciences*, 101, 313-325.
- Vergilio, C. S., Lacerda, D., da Silva Souza, T., de Oliveira, B. C. V., Fioresi, V. S., de Souza, V. V., ... & de Rezende, C. E. (2021). Immediate and long-term impacts of one of the worst mining tailing dam failure worldwide (Bento Rodrigues, Minas Gerais, Brazil). *Science of the Total Environment*, 756, 143697.
- Vergilio, C. S., Lacerda, D., Oliveira, B. C. V. D., Sartori, E., Campos, G. M., Pereira, A. L. D. S., ... & Rezende, C. E. D. (2020). Metal concentrations and biological effects from one of the largest mining disasters in the world (Brumadinho, Minas Gerais, Brazil). *Scientific reports*, 10(1), 5936.
- Vieira, C. D., Oliveira, D. F. C., Frota, M. N., Viana, L. Q., Farias, C. A. S., Gonçalves, R. A., & Godoy, J. M. (2022). Siltation processes and metal sediment profiles in a hydroelectric power plant reservoir in the Paraíba do Sul River Basin, Southeastern Brazil. *Environmental Earth Sciences*, 81(523).
- Wanderley, C. V., Godoy, J. M., Godoy, M. L. D., Rezende, C. E., Lacerda, L. D., Moreira, I., & Carvalho, Z. L. (2014). Evaluating sedimentation rates in the estuary and shelf region of the Paraíba do Sul River, Southeastern Brazil. *Journal of the Brazilian Chemical Society*, 25, 50-64.
- Wu, J., Cheng, S. P., He, L. Y., Wang, Y. C., Yue, Y., Zeng, H., & Xu, N. (2023). Assessing water quality in the Pearl River for the last decade based on clustering: Characteristic, evolution and policy implications. *Water Research*, 244, 120492.
- Yadav, M. L., & Roychoudhury, B. (2018). Handling missing values: A study of popular imputation packages in R. *Knowledge-Based Systems*, 160, 104-118

Supplementary Material

Devido ao extensivo material suplementar (mais de 100 páginas de arquivo), o material ficará disponível no link abaixo.

Link: [Material Suplementar Capítulo 3](#)

CONSIDERAÇÕES FINAIS

O desenvolvimento desta tese contribuiu para ampliar a compreensão dos efeitos das mudanças no uso e ocupação do solo sobre a dinâmica biogeoquímica, a contaminação ambiental e a qualidade de ecossistemas aquáticos tropicais. Ao integrar abordagens biogeoquímicas, ecotoxicológicas e de monitoramento ambiental em duas bacias hidrográficas brasileiras com históricos contrastantes de ocupação, o Rio Caeté (RC, Amazônia) e o Rio Paraíba do Sul (RPS, Mata Atlântica), este estudo permitiu estabelecer conexões entre as pressões antrópicas, os processos de transporte e acúmulo de contaminantes e seus reflexos sobre os sedimentos, a biota bentônica e a qualidade da água.

O primeiro capítulo demonstrou que a composição isotópica e elementar da matéria orgânica sedimentar, associada à distribuição de poluentes orgânicos persistentes, constitui um conjunto robusto de marcadores biogeoquímicos para rastrear os efeitos do uso e ocupação do solo em ecossistemas costeiros tropicais. As assinaturas biogeoquímicas contrastantes observadas entre as bacias evidenciam diferentes trajetórias de ocupação e degradação ambiental. Enquanto o RC ainda preserva características predominantemente naturais, com os manguezais desempenhando papel fundamental na retenção de matéria orgânica e contaminantes provenientes da bacia, o RPS apresenta uma assinatura geoquímica fortemente influenciada por décadas de urbanização, industrialização e lançamento de efluentes domésticos.

O segundo capítulo avançou na compreensão dos riscos ecotoxicológicos associados à exposição da biota bentônica a misturas complexas de contaminantes sedimentares. A combinação de bioensaios crônicos com *Nitokra* sp. e modelagem estatística de misturas permitiu identificar os principais grupos de contaminantes associados à redução da produção reprodutiva, evidenciando que PCBs, pesticidas organoclorados e PAHs de alto peso molecular exercem influência mais relevante do que a avaliação individual dos compostos seria capaz de revelar. Esses resultados demonstram que a toxicidade dos sedimentos decorre da ação conjunta de múltiplos contaminantes, reforçando as limitações de abordagens baseadas exclusivamente em concentrações individuais e destacando a necessidade de incorporar avaliações de mistura aos processos de gestão e regulamentação ambiental.

O terceiro capítulo ampliou a escala espacial e temporal da investigação ao avaliar uma década de monitoramento da qualidade da água na bacia do RPS. Os resultados evidenciaram uma deterioração progressiva de diversos parâmetros físicos, químicos e biológicos, indicando que as pressões decorrentes da urbanização, da atividade industrial e do uso intensivo do solo continuam comprometendo a qualidade ambiental da bacia. A persistência dessas alterações reforça a importância de programas contínuos de monitoramento e da integração entre instituições responsáveis pela gestão dos recursos hídricos para subsidiar ações preventivas e estratégias de recuperação ambiental.

De forma integrada, os resultados obtidos demonstram que as mudanças no uso e ocupação do solo desencadeiam uma sequência de processos interligados que se inicia na modificação da dinâmica da matéria orgânica e do transporte de contaminantes ao longo das bacias hidrográficas, culminando no acúmulo dessas substâncias nos sedimentos estuarinos, em efeitos ecotoxicológicos sobre a biota bentônica e na degradação da qualidade da água. Dessa forma, esta tese evidencia que a compreensão dos impactos antrópicos em ecossistemas aquáticos tropicais depende de uma abordagem integrada, capaz de conectar processos que ocorrem em diferentes compartimentos ambientais e escalas espaciais.

Além das contribuições para o entendimento dos processos ambientais, este trabalho também apresenta implicações metodológicas relevantes. A integração entre marcadores isotópicos, contaminantes químicos, bioensaios ecotoxicológicos, modelagem estatística de misturas e séries históricas de monitoramento demonstrou ser uma estratégia eficiente para identificar tanto as fontes de contaminação quanto os contaminantes efetivamente associados aos efeitos biológicos observados. Essa abordagem pode ser aplicada em outras bacias hidrográficas tropicais submetidas a diferentes cenários de uso e ocupação do solo, contribuindo para avaliações ambientais mais robustas e ecologicamente representativas.

Por fim, embora esta tese tenha avançado na identificação dos principais processos que conectam as pressões antrópicas aos impactos ambientais em sistemas aquáticos tropicais, permanecem desafios importantes. Estudos futuros devem investigar os mecanismos de interação entre diferentes classes de contaminantes, incorporar outros grupos de compostos emergentes e expandir a aplicação de abordagens baseadas em misturas para diferentes organismos e níveis

de organização biológica. Da mesma forma, o fortalecimento de programas de monitoramento de longo prazo será essencial para compreender a evolução dos impactos ambientais frente às mudanças no uso do solo e às alterações climáticas.

Em conjunto, os resultados apresentados reforçam que a gestão eficiente de bacias hidrográficas tropicais deve considerar a integração entre processos biogeoquímicos, qualidade ambiental e respostas biológicas. Ao estabelecer essa conexão de forma quantitativa e em múltiplas escalas, esta tese fornece subsídios científicos para o aprimoramento de programas de monitoramento, avaliação de risco e conservação de ecossistemas costeiros tropicais.

REFERENCIAS BIBLIOGRÁFICAS

- Barhoumi, B., Beldean-Galea, M. S., Al-Rawabdeh, A. M., Roba, C., Martonos, I. M., Bălc, R., Kahlaoui, M., Touil, S., Tedetti, M., Driss, M. R., & Baciú, C. (2019). Occurrence, distribution and ecological risk of trace metals and organic pollutants in surface sediments from a Southeastern European river (Someșu Mic River, Romania). *Science of The Total Environment*, 660, 660–676. <https://doi.org/10.1016/j.scitotenv.2018.12.428>
- Bayen, S. (2012). Occurrence, bioavailability and toxic effects of trace metals and organic contaminants in mangrove ecosystems: A review. *Environment International*, 48, 84–101. <https://doi.org/10.1016/j.envint.2012.07.008>
- Bunting, P., Rosenqvist, A., Hilarides, L., Lucas, R.M., Thomas, N., Tadono, T., Worthington, T.A., Spalding, M., Murray, N.J., Rebelo, L.-M., 2022. Global mangrove extent change 1996–2020: global mangrove watch version 3.0. *Remote Sens* 14, 3657. <https://doi.org/10.3390/rs14153657>.
- Call, M., Santos, I. R., Dittmar, T., de Rezende, C. E., Asp, N. E., & Maher, D. T. (2019). High pore-water derived CO₂ and CH₄ emissions from a macro-tidal mangrove creek in the Amazon region. *Geochimica et Cosmochimica Acta*, 247, 106–120. <https://doi.org/10.1016/j.gca.2018.12.029>
- UNDRR-UNITED NATIONS OFFICE FOR DISASTER RISK REDUCTION (UNDRR). Loss of mangroves. Geneva: UNDRR, 2023. Disponível em: <https://www.undrr.org/understanding-disaster-risk/terminology/hips/en0207>. Acesso em: 31 maio 2026.
- Doong, R., Lee, S., Lee, C., Sun, Y., & Wu, S. (2008). Characterization and composition of heavy metals and persistent organic pollutants in water and estuarine

- sediments from Gao-ping River, Taiwan. *Marine Pollution Bulletin*, 57(6–12), 846–857. <https://doi.org/10.1016/j.marpolbul.2007.12.015>
- Gerson, J. R. (2025). The role of contaminant risk landscapes in the Anthropocene. *Frontiers in Ecology and the Environment*, 23(3). <https://doi.org/10.1002/fee.2818>
- Horowitz, A. J. (1991). *A primer on sediment-trace element chemistry* (2nd ed., Open-File Report 91-76). U.S. Geological Survey.
- ICMBio - Instituto Chico Mendes de Conservação da Biodiversidade. (2018). *Atlas dos manguezais do Brasil*. ICMBio. ISBN 978-85-61842-75-8
- Jones, K. C., & de Voogt, P. (1999). Persistent organic pollutants (POPs): state of the science. *Environmental Pollution*, 100(1–3), 209–221. [https://doi.org/10.1016/s0269-7491\(99\)00098-6](https://doi.org/10.1016/s0269-7491(99)00098-6)
- Keil, A. P., Buckley, J. P., O'Brien, K. M., Ferguson, K. K., Zhao, S., & White, A. J. (2020). A Quantile-Based g-Computation Approach to Addressing the Effects of Exposure Mixtures. *Environmental Health Perspectives*, 128(4). <https://doi.org/10.1289/ehp5838>
- Lacerda, L. D., Carvalho, C. E. V., Tanizaki, K. F., Ovalle, A. R. C., & Rezende, C. E. (1993). The Biogeochemistry and Trace Metals Distribution of Mangrove Rhizospheres. *Biotropica*, 25(3), 252. <https://doi.org/10.2307/2388783>
- Lotufo, G., & Abessa, D., 2002. Testes de toxicidade com sedimento total e água intersticial estuarinos utilizando copépodes bentônicos. *Métodos em Ecotoxicologia Marinha - Aplicações no Brasil*.
- Martinelli, L. A., Nardoto, G. B., Soltangheisi, A., Reis, C. R. G., Abdalla-Filho, A. L., Camargo, P. B., Domingues, T. F., Faria, D., Figueira, A. M., Gomes, T. F., Lins, S. R. M., Mardegan, S. F., Mariano, E., Miatto, R. C., Moraes, R., Moreira, M. Z., Oliveira, R. S., Ometto, J. P. H. B., Santos, F. L. S., ... Vieira, S. A., 2020. Determining ecosystem functioning in Brazilian biomes through foliar carbon and nitrogen concentrations and stable isotope ratios. *Biogeochemistry*, 154(2), 405–423. <https://doi.org/doi.org/10.1007/s10533-020-00714-2>
- Molisani, M. M., Salomão, M. S. B., Ovalle, A. R. C., Rezende, C. E., Lacerda, L. D., & Carvalho, C. E. V. (1999). Heavy metals in sediments of the Lower Paraíba do Sul River and Estuary, R.J., Brazil. *Bulletin of Environmental Contamination and Toxicology*, 63(6), 682–690.

- Oliveira, A. H. B., Cavalcante, R. M., Duaví, W. C., Fernandes, G. M., Nascimento, R. F., Queiroz, M. E. L. R., & Mendonça, K. V., 2016. The legacy of organochlorine pesticide usage in a tropical semi-arid region (Jaguaribe River, Ceará, Brazil): Implications of the influence of sediment parameters on occurrence, distribution and fate. *Science of The Total Environment*, 542, 254–263. <https://doi.org/10.1016/j.scitotenv.2015.10.058>
- Ovalle, A. R. C., Silva, C. F., Rezende, C. D., Gatts, C. E. N., Suzuki, M. S., & Figueiredo, R. O. (2013). Long-term trends in hydrochemistry in the Paraíba do Sul River, southeastern Brazil. *Journal of hydrology*, 481, 191-203.
- Pereira, L. C. C., Sousa, N. S. S., Rodrigues, L. M. S., Monteiro, M. C., Silva, S. R. S., Oliveira, A. R. G., Dias, A. B. B., & Costa, R. M., 2021. Effects of the lack of basic public sanitation on the water quality of the Caeté River estuary in northern Brazil. *Ecohydrology and Hydrobiology*, 21(2), 299–314. <https://doi.org/10.1016/j.ecohyd.2020.12.003>
- Pfeiffer, W. C., Fiszman, M., Malm, O., & Azcue, J. M., 1986. Heavy metal pollution in the Paraíba do sul river. *The Science of the Total Environment*, 58, 73–79. [https://doi.org/doi.org/10.1016/0048-9697\(86\)90077-X](https://doi.org/doi.org/10.1016/0048-9697(86)90077-X)
- Quaresma, V. S., Aguiar, V. M. C., Bastos, A. C., Oliveira, K. S., Vieira, F. V., Sá, F., & Baptista Neto, J. A. (2021). The impact of trace metals in marine sediments after a tailing dam failure: the Fundão dam case (Brazil). *Environmental Earth Sciences*, 80(17). <https://doi.org/10.1007/s12665-021-09817-x>
- Serafim, T. S. G., Almeida, M. G., Thouzeau, G., Michaud, E., Niggemann, J., Dittmar, T., Seidel, M., & Rezende, C. E., 2023. Land-use changes in Amazon and Atlantic rainforests modify organic matter and black carbon compositions transported from land to the coastal ocean. *Science of The Total Environment*, 878, 162917. <https://doi.org/10.1016/j.scitotenv.2023.162917>
- Serafim, T. S. G., Simon, H., Niggemann, J., Rüger, C. P., Zimmermann, R., Mairon, T. N. L., Waniek, J. J., & Osterholz, H. (2026). Differential Drivers of Thermogenic and Bulk Dissolved Organic Matter in the Indian Ocean. *Global Biogeochemical Cycles*, 40(5). <https://doi.org/10.1029/2025gb008975>
- Silva, A. C. F. S., de Santana, C. O., dos Santos Vergilio, C., and de Jesus, T. B. (2023). Distribution, toxicity and bioaccumulation of trace metals in environmental matrices of an estuary in a protected area. *Marine Pollution Bulletin*, 191, 114928. <https://doi.org/10.1016/j.marpolbul.2023.114928>
- Souza, L. M. E., Vidal, L. O., Lima, V. S., Rezende, C. E., and Suzuki, M. S. (2026). Long-Term Variation of Hydrochemical Parameters in a Medium-Sized Tropical

River: Effects of a Severe Drought. *Journal of the Brazilian Chemical Society*.
<https://doi.org/10.21577/0103-5053.20260033>

- Sreedevi, M. A., & Harikumar, P. S. (2023). Occurrence, distribution, and ecological risk of heavy metals and persistent organic pollutants (OCPs, PCBs, and PAHs) in surface sediments of the Ashtamudi wetland, south-west coast of India. *Regional Studies in Marine Science*, 64, 103044. <https://doi.org/10.1016/j.rsma.2023.103044>
- Swain, C. K. (2024). Environmental pollution indices: a review on concentration of heavy metals in air, water, and soil near industrialization and urbanisation. *Discover Environment*, 2(1). <https://doi.org/10.1007/s44274-024-00030-8>
- Tanaka, Y., Minggat, E., & Roseli, W. (2021). The impact of tropical land-use change on downstream riverine and estuarine water properties and biogeochemical cycles: a review. *Ecological Processes*, 10(1). <https://doi.org/10.1186/s13717-021-00315-3>
- Thornton, S. F., and McManus, J., 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: Evidence from the Tay Estuary, Scotland. *Estuarine, Coastal and Shelf Science*, 38, 219-233.
- Turubanova, S., Potapov, P. V., Tyukavina, A., & Hansen, M. C. (2018). Ongoing primary forest loss in Brazil, Democratic Republic of the Congo, and Indonesia. *Environmental Research Letters*, 13(7), 074028. <https://doi.org/10.1088/1748-9326/aacd1c>
- Vergilio, C. S., Lacerda, D., da Silva Souza, T., de Oliveira, B. C. V., Fioresi, V. S., de Souza, V. V., da Rocha Rodrigues, G., de Araujo Moreira Barbosa, M. K., Sartori, E., Rangel, T. P., de Almeida, D. Q. R., de Almeida, M. G., Thompson, F., & de Rezende, C. E. (2021). Immediate and long-term impacts of one of the worst mining tailing dam failure worldwide (Bento Rodrigues, Minas Gerais, Brazil). *Science of The Total Environment*, 756, 143697. <https://doi.org/10.1016/j.scitotenv.2020.143697>
- Ward, D. J., Perez-Landa, V., Spadaro, D. A., Simpson, S. L., & Jolley, D. F. (2011). An Assessment of Three Harpacticoid Copepod Species for Use in Ecotoxicological Testing. *Archives of Environmental Contamination and Toxicology*, 61(3), 414–425. <https://doi.org/10.1007/s00244-011-9646-2>

Yu, L., Liu, W., Wang, X., Ye, Z., Tan, Q., Qiu, W., Nie, X., Li, M., Wang, B., & Chen, W. (2022). A review of practical statistical methods used in epidemiological studies to estimate the health effects of multi-pollutant mixture. *Environmental Pollution*, 306, 119356. <https://doi.org/10.1016/j.envpol.2022.119356>