



**UNIVERSIDADE ESTADUAL DO SUDOESTE DA BAHIA**  
**PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA**  
**ÁREA DE CONCENTRAÇÃO: FITOTECNIA**

**SOIL CARBON STOCK AND ECOLOGICAL RISK FROM  
TRACE ELEMENTS IN CACAO AGROFORESTRY  
SYSTEMS - CABRUCO IN THE COUNTRY'S MAIN  
PRODUCING REGION, NORTHEAST BRAZIL**

**TATIANA REIS DOS SANTOS BASTOS**

**VITÓRIA DA CONQUISTA**  
**BAHIA – BRASIL**  
**2025**

**TATIANA REIS DOS SANTOS BASTOS**

**SOIL CARBON STOCK AND ECOLOGICAL RISK FROM TRACE  
ELEMENTS IN CACAO AGROFORESTRY SYSTEMS - CABRUCÁ  
IN THE COUNTRY'S MAIN PRODUCING REGION, NORTHEAST  
BRAZIL**

Thesis presented to the State University of  
Southwest Bahia, as part of the requirements of  
the Graduate Program in Agronomy, area of  
concentration in Crop Science, to obtain the  
title of Doctor.

Advisor: Prof. Dr<sup>o</sup>. Cácio Luiz Boechat

Coadvisor: Prof. Dr<sup>o</sup>. João Carlos Medeiros

VITÓRIA DA CONQUISTA  
BAHIA – BRASIL  
2025

B331s

Bastos, Tatiana Reis dos Santos..

Soil carbon stock and ecological risk from trace elements in cacaoagroforestry systems – cabruca in the country's main producing region, northeast Brazil / Tatiana Reis dos Santos Bastos, 2025.

114f. ; il.

Orientador (a): Dr. Cácio Luiz Boechat.

Tese (doutorado) – Universidade Estadual do Sudoeste da Bahia, Programa de Pós-Graduação em Agronomia, Área de concentração em Fitotecnia. Vitória da Conquista, 2025.

Inclui referências.

1. Carbon stock. 2. Heavy metals. 3. Quality reference values. 4. Soil quality. 5. Environmental monitoring. I. Boechat, Cácio Luiz. II. Universidade Estadual do Sudoeste da Bahia, Programa de Pós - Graduação em Agronomia. III. T.

CDD 630

*Catálogo na fonte: Karolyne Alcântara Profeta – CRB 5/2134*  
UESB - Campus Vitória da Conquista – BA



**UNIVERSIDADE ESTADUAL DO SUDOESTE DA BAHIA  
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA  
Área de Concentração em Fitotecnia**

**Campus de Vitória da Conquista, BA**

**DECLARAÇÃO DE APROVAÇÃO**

**TÍTULO: “SOIL CARBON STOCK AND ECOLOGICAL RISK FROM TRACE ELEMENTS IN COCOA AGROFORESTRY SYSTEMS - CABRUCÁ IN THE COUNTRY’S MAIN PRODUCING REGION, NORTHEAST BRAZIL”.**

**AUTOR (A): Tatiana Reis dos Santos Bastos**

**Aprovada como parte das exigências para obtenção do Título de DOUTORA EM AGRONOMIA, ÁREA DE CONCENTRAÇÃO EM FITOTECNIA, pela seguinte Banca Examinadora:**



**CACIO LUIZ BOECHAT**  
Data: 04/08/2025 10:24:06-0300  
Verifique em <https://validar.iti.gov.br>

Cácio Luiz Boechat, D.Sc. (UESB)

Documento assinado digitalmente



**JOAO CARLOS MEDEIROS**  
Data: 31/07/2025 22:59:13-0300  
Verifique em <https://validar.iti.gov.br>

João Carlos Medeiros, D.Sc. (UFSB)

Documento assinado digitalmente



**PATRICIA ANJOS BITTENCOURT BARRETO GAR**  
Data: 31/07/2025 15:39:31-0300  
Verifique em <https://validar.iti.gov.br>

Patrícia Anjos Bittencourt Barreto-Garcia, D.Sc. (UESB)

Documento assinado digitalmente



**MARIA EUGENIA ORTIZ ESCOBAR**  
Data: 31/07/2025 15:11:53-0300  
Verifique em <https://validar.iti.gov.br>

Maria Eugênia Ortiz Escobar, D.Sc. (UFC)

Documento assinado digitalmente



**THOMAS VINCENT GLOAGUEN**  
Data: 01/08/2025 14:56:16-0300  
Verifique em <https://validar.iti.gov.br>

Thomas Vicent Gloaguen, D.Sc. (UFRB)

Data de realização: 31 de julho de 2025.

Estrada do Bem Querer, Km 4, CEP 45031-900, Caixa Postal 95, Vitória da Conquista, Bahia, Brasil  
Telefone: (77) 3425-9383, e- mail: [ppgagronomia@uesb.edu.br](mailto:ppgagronomia@uesb.edu.br)

## DEDICATION

To my greatest loves: Thayla Bastos (daughter), Flávio Bastos (husband), Leonice and Reinaldo (parents), Ariana (sister), Maria de Lourdes (grandmother).

I dedicate.

## ACKNOWLEDGEMENTS

My first gratitude is to God, for leading me with light, wisdom, and serenity at every stage of this arduous journey. His constant presence was my foundation, that's why I'm here. I thank God not only for the strength, but for the gift of life and for the constant acquisition of knowledge, which has transformed me along this journey.

To my husband, Flávio, my companion in all moments, thank you for walking by my side with love, patience, and generosity. Your unconditional support was essential at every step, and every achievement carries your presence and dedication.

To my daughter, Thayla, my greatest source of inspiration. It is for you and because of you that I pursued this path with even greater determination. May this work serve as an example that everything is possible when there are dreams, focus, and courage. To my parents, Leonice and Reinaldo, thank you for all your love, example, effort, and for always believing in my potential. Every step taken here carries a piece of the history that you helped me build.

To my grandmother, Maria de Lourdes, my eternal love and gratitude. Her strength, wisdom, and affection were, and always will be, a reference for me.

To my family as a whole, thank you for every gesture of encouragement, for every word of support and for being by my side with so much affection and pride. I carry with me the values you taught me: the importance of fighting with dignity and persistence.

To the friends who cheered for me, even from afar, thank you for sharing in my joy. Every message, every conversation, every affection remains in my heart.

To my advisor, Professor Cácio Luiz Boechat, thank you for your trust, for your attentive listening, and for your firm yet generous guidance. To my co-advisor, Professor João Carlos Medeiros, I am deeply grateful for the technical support, partnership and for contributing with his experience to strengthen this work.

I would also like to thank the team at the UESB, UFPI, UFRPE, and UFSB laboratories for their dedicated and excellent analyses, which were fundamental to the development of this research.

To the UESB transport sector team, my sincere gratitude for the collaboration in all field trips, and especially to the driver for their professionalism and support during each journey.

To my colleagues Geison and Ivan, students at UFSB, my deep gratitude for accompanying me on all the field trips to collect cacao soils, with commitment, partnership and friendship.

To the UESB Graduate team, my sincere gratitude for the support, guidance, and reception throughout the journey. Every service and every care contributed to making this journey possible.

To the professors who shaped my academic career, thank you for your teaching and encouragement. Their contributions were essential and continue with me beyond the university.

To my university, and to the institutions that welcomed me, especially during the exchange in Germany, my gratitude for expanding my horizons and strengthening my training as both a researcher and citizen.

I would like to thank CAPES – Coordination for the Improvement of Higher Education Personnel – for the financial support that was essential for the development of research activities, academic training and my scientific consolidation, contributing significantly to the advancement of knowledge and to my professional career.

Finally, I affirm that I have traveled this entire path with a light heart and an optimistic mind. I never thought about giving up. I always believed it was possible — and it was. This moment is the celebration of a journey built with love, faith, science, and courage.

Gratitude!

## EPIGRAPH

I refuse... as long as the sun exists, the source of energy for planet Earth, I refuse to think that there is no hope of life on this Gaia;

While, at the dawn of a new day, I feel the freshness of the serene, I refuse to think that there is only polluted air;

As long as there is the murmur of crystal-clear water that meanders over the rocks, I refuse to think that there are only dead rivers;

While on earth there is still a grain for daily bread, I refuse to think that there are only exhausted soils;

As long as there is the immense sea embracing the white sand,

I refuse to think that there are only beaches "unsuitable for bathing"; As long as the rich Amazon Rainforest exists,

I refuse to think about the extinction of flora and fauna;

As long as there is a feeling of fraternity in a few fighting for all, I refuse to think that it is impossible to change the heart of man; As long as there is Science and a scientist to research,

I refuse to think that environmental problems are insoluble; As long as mothers bear children and love exist,

I refuse to think that man is capable of self-destruction; As long as dialogue exists,

I refuse to think that hatred is the spring of the world; As long as there are educators,

I refuse to think that we cannot offer a more promising future to youth; As long as thousands of young people yearn for a more just world,

I refuse to think that we cannot transform the egocentric society; As long as work and solidarity exist,

I refuse to think that we cannot reduce the misery of peoples; As long as you have strength,

I refuse to think that together we won't fight for a better tomorrow...

..... for all mankind.

For we are nature itself, thinking nature.

Helmut Tropicmair

## GENERAL ABSTRACT

**BASTOS, T. R. S. Soil carbon stock and ecological risk from trace elements in cacao agroforestry systems - cabruca in the country's main producing region, Northeast Brazil, 2025.** 114 p. (Thesis: Doctor Science in Agronomy; Area of Concentration: Crop Science) \*

The quantification of soil carbon and the evaluation of trace elements (TEs) in tropical ecosystems are fundamental to understanding the sustainability of production systems and their contribution to climate change mitigation. In this context, the cacao-cabruca (AFS-C) and native forest (NF) agroforestry systems are strategic environments for quantifying carbon stocks, defining soil quality reference values, and assessing potential ecological risks. The cacao region of Bahia, the largest cacao producer in Brazil, holds agricultural, historical, and socio-environmental importance. It has also undergone recent landscape driven by the adoption of SAFs within the Atlantic Forest. In this study, total soil carbon concentrations were determined in cacao-cabruca agroforestry systems (AFS-C) (n = 188) and native forest (NF) (n = 152), at depths of 0–10, 10–20, 20–40 and 40–60 cm, in addition to litter quantification. Reference values for the quality of trace elements (TEs) were also established in surface soils (0.00–0.20 m) of natural areas (n = 38) of the Atlantic Forest in the cacao-growing region of Bahia. Pollution, enrichment, and ecological and individual risk indices of selected TEs in AFS-C were calculated. Carbon quantification was performed by elemental analyzer, while TE extraction followed the USEPA 3051A protocol, with analyses conducted by inductively coupled plasma optical emission spectroscopy (ICP-OES). Litter samples were collected from 0.0625 m<sup>2</sup> quadrats using stratified random sampling. For data analysis, multivariate statistics and graphical visualizations were applied. The results indicated an average carbon stock of 113.98 Mg C ha<sup>-1</sup> in the soil profile (0–60 cm) of AFS-C and an average litter biomass of 7.8 Mg ha<sup>-1</sup>. This environmental complexity simultaneously influences the natural concentrations, the distribution of trace elements in the soil, and the geochemical patterns, resulting in specific and distinct TE concentrations, in the following order: Fe > Ti > Ba > V > Cr > Zn > Cu > Co > Ni > Pb > Cd > Sb > Mo, of natural geogenic origin. Based on the ecological risk indexes, the analyzed region presents pollution levels ranging from minimal to moderate, indicating relatively controlled environmental impact of trace elements. The results confirm AFS-C as relevant in carbon sequestration, environmental conservation, and trace element monitoring in tropical soils.

**Keywords:** Carbon stock, Heavy metals, Quality reference values, Soil quality, Environmental monitoring

---

\* **Advisor:** Prof<sup>o</sup>. Dr<sup>o</sup>. Cácio Luiz Boechat, UFPI and **Coadvisor:** Prof<sup>o</sup>. Dr<sup>o</sup>. João Carlos Medeiros, UFSB.

## RESUMO GERAL

BASTOS, T. R. S. **Estoque de carbono no solo e risco ecológico de elementos-traço em sistemas agroflorestais de cacau - cabruca na principal região produtora do país, Nordeste do Brasil**, 2025. 114 p. (Tese: Doutorado em Agronomia; Área de Concentração: Fitotecnia) \*

A quantificação do carbono no solo e a avaliação de elementos-traço (ETs) em ecossistemas tropicais são fundamentais para compreender a sustentabilidade de sistemas produtivos e sua contribuição à mitigação das mudanças climáticas. Nesse contexto, os sistemas agroflorestais de cacau-cabruca (SAF-C) e floresta nativa (FN) configuram-se como ambientes estratégicos para quantificar estoques de carbono, definir valores de referência de qualidade do solo e avaliar potenciais riscos ecológicos. A região cacauceira da Bahia, maior produtora de cacau do Brasil, reúne importância agrícola, histórica e socioambiental, além de apresentar transformações recentes na paisagem, impulsionadas pela adoção de SAFs em meio à Mata Atlântica. Neste estudo foram determinadas concentrações de carbono total no solo em sistemas agroflorestais de cacau-cabruca - SAF-C (n = 188) e floresta nativa - FN (n = 152), nas profundidades de 0–10, 10–20, 20–40 e 40–60 cm, além da quantificação da serapilheira. Também foram estabelecidos valores de referência de qualidade dos elementos-traço (ETs) em solos superficiais (0,00–0,20 m) de áreas naturais (n = 38) da Floresta Atlântica na região cacauceira do estado da Bahia. Foram calculados índices de poluição, enriquecimento e de riscos ecológico e individual de alguns ETs em SAF-C. A quantificação do carbono foi realizada por analisador elementar, enquanto a extração dos ETs seguiu o protocolo USEPA 3051A, com análises conduzidas por espectroscopia de emissão óptica com plasma acoplado indutivamente (ICP-OES). Amostras de serapilheira foram coletadas em quadrado de 0,0625 m<sup>2</sup> por amostragem aleatória estratificada. Para a análise dos dados, empregaram-se estatística multivariada e visualizações gráficas. Os resultados indicaram estoque médio de carbono de 113,98 Mg C ha<sup>-1</sup> no perfil do solo (0–60 cm) nos SAF-C e biomassa média da serapilheira de 7,8 Mg ha<sup>-1</sup>. Essa complexidade ambiental ao mesmo tempo influencia nas concentrações naturais, na distribuição dos elementos-traço no solo e nos padrões geoquímicos observados, resultando em concentrações específicas e distintas de ETs, seguindo a ordem: Fe > Ti > Ba > V > Cr > Zn > Cu > Co > Ni > Pb > Cd > Sb > Mo, de origem geogênica natural. Com base nos índices de risco ecológico, a região analisada apresenta níveis de poluição entre as categorias de mínima a moderada, indicando impacto ambiental dos elementos-traço relativamente controlado. Os resultados confirmam os SAF-C como relevantes no sequestro de carbono, na conservação ambiental e no monitoramento de elementos-traço nos solos tropicais.

**Palavras-chave:** Estoque de carbono, Metais pesados, Valores de referência e qualidade, Risco ecológico, Monitoramento ambiental

---

\* **Orientador:** Prof<sup>o</sup>. Dr<sup>o</sup>. Cácio Luiz Boechat, UFPI e **Coorientador:** Prof<sup>o</sup>. Dr<sup>o</sup>. João Carlos Medeiros, UFSB

## LIST OF FIGURES

Figure 1.1 - Location of the cacao-growing region of Bahia (a), where the cacao agroforestry system – AFS-C and native forest – NF are established, (b) elevation, (c) geology, (d) soil classes and (e) climate of the cacao-growing region of Bahia, northeastern Brazil.....	27
Figure 1.2 - Spatial arrangement of the agroforestry system of cacao and native forest, located in the cacao-growing region of Bahia, northeastern Brazil .....	29
Figure 1.3 - Differences between (a) total organic carbon – TOC per depth and (b) soil carbon Stock-C, (c) cumulative soil carbon stock, (d) nitrogen – TN per depth and (e) carbon-nitrogen ratio – C/N per depth in agroforestry system of cacao - AFS-C and native forest – NF, located in the cacao region of Bahia, northeastern Brazil. Error bars represent the standard error of the mean. Different letters indicate significant differences between the groups, using Mann-Whitney ( $p < 0.05$ ).....	36
Figure 1.4 - Fractions of litter (a) from AFS-C and NF (b), total litter AFS-C and NF, located in the cacao-growing region of Bahia, northeastern Brazil.....	38
Figure 1.5 - Analysis of Randon Forest referring to the degree of importance of soil fertility and granulometry attributes in total soil carbon - TOC, for (a) AFS-C and (b) NF, located in the cacao-growing region of Bahia, northeastern Brazil. Based on these attributes: m (aluminum saturation), pH, Mg, Silt, C/N N (carbon/nitrogen ratio), V (base saturation), Zn, Al, Cu, Mn, SB (sum of bases), sand, P, clay, BD (bulk density), Fe, H+Al (potential acidity), K, Ca, CEC (cation exchange capacity) and TN (total nitrogen).....	41
Figure 1.6 - Linear regression analyses showing the relationships between total soil carbon - TOC and TN in AFS-C and NF, In the layers:(a) 0-10 cm, (b)10-20 cm, (c) 20-40 cm and (d) 40-60 cm, located in the cacao-growing region of Bahia, northeastern Brazil .....	42
Figure 1.7 - Biplot from (a) principal component analysis and (b) hierarchical clustering (Ward's method) of soil fertility and granulometry attributes; m - aluminum saturation, pH, Mg, silt, C/N -carbon/nitrogen ratio, V - base saturation, Zn, Al, Cu, Mn, SB - sum of bases, sand, P, clay, BD - bulk density, Fe, H+Al - potential acidity K, Ca, CEC - cation exchange capacity and TN - total nitrogen in AFS-C and NF, located in the cacao region of the state of Bahia, northeastern Brazil.....	44
Figure 2.1 - Location map of the study area. (a) sampling points in the Atlantic Forest, located in the southern region of Bahia State, Brazil, (b) elevation, (c) geology, and (d) soil types .....	58
Figure 2.2 - Spearman correlation matrix between trace elements and soil attributes (n = 38), significant correlations ( $r \geq 0.70$ ). .....	67
Figure 3.1 - Location of the study areas (a), altitude (b), soil classes (c), and geology (d) of soils under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia, northeastern Brazil.....	85

Figure 3.2 - Spearman's correlation analysis between environmental factors and trace elements concentrations in soils from the cacao-growing region of Bahia, northeastern Brazil. Elev = Elevation; Class = Soil class; Geo = Geology; Clim = Climate. (\*\*\*)  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$  indicate statistically significant correlations) .....94

Figure 3.3 - Biplot from Principal Component Analysis (PCA) applied to soil attributes and trace element concentrations along the soil profile. m (%) = aluminum saturation; C/N = carbon-to-nitrogen ratio; V = base saturation; SB = sum of bases; H+Al = potential acidity; TOC = total organic carbon; TN = total nitrogen. Colored ellipses represent the dispersion of sample points within each soil layer in the study areas located in the cacao-growing region of Bahia.....96

Figure 3.4 - Vertical distribution of trace element concentrations and Soil Quality Reference Values (QRVs) in soils under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia, northeastern Brazil..... 99

Figure 3.5- Individual values of (a) Geoaccumulation Index (Igeo), (b) Contamination Factor (CF), and (c) Enrichment Factor (EF) for trace elements along the soil profile under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia, northeastern Brazil. The dotted line represents the threshold for considerable risk. Colored bars indicate individual values; black bars represent the standard error ..... 101

Figure 3.6 - Values of (a) Individual Ecological Risk Index (ER<sub>i</sub>) and (b) Potential Ecological Risk Index (PERI) for trace elements along the soil profile in soils under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia. The dotted line represents the threshold for considerable risk..... 103

## LIST OF TABLES

Table 1.1 - Soil attributes of AFS-C (n = 47) and NF (n = 38), located in the cacao-growing region of Bahia, northeastern Brazil.....	33
Table 1.2 - Factor analysis (PCA) of soil chemical and granulometric properties in agroforestry systems of cacao and native forests, located in the cacao-growing region of Bahia, northeastern Brazil.....	45
Table 2.1 -Recovery rates of trace elements (TEs) in the certified soil sample SRM 2709 - San Joaquin.....	62
Table 2.2 - Chemical attributes and granulometry of soil samples under Atlantic Forest, located in the southern region of Bahia (n = 38).....	63
Table 2.3 - Average concentrations of TEs (mg kg <sup>-1</sup> , Fe in g kg <sup>-1</sup> , dry soil) in Atlantic Forest soils (0.0–0.20 m), located in the southern region of Bahia, Brazil (n = 38), compared with data compiled in national and international literature.....	65
Table 2.4 - Factor analysis between the original variables and the principal components of chemical elements, granulometry, and TEs of soils under the Atlantic Forest, located in the southern region of Bahia.....	68
Table 2.5 - Descriptive statistics and soil quality reference values (QRVs) for TEs in soils under the Atlantic Forest, located in the southern region of Bahia, Brazil.....	70
Table 3.1 - Categories of the geoaccumulation index (I <sub>geo</sub> ), contamination factor (FC), enrichment factor (EF), individual ecological risk (ER <sub>i</sub> ) and potential ecological risk index (PERI).....	90
Table 3.2 - Soil attributes in the agroforestry system in the cacao-growing region of Bahia, northeastern Brazil (n = 50).....	91
Table 3.3 - Descriptive statistics of trace elements in soils affected by human activities in the cacao-growing region of Bahia, northeastern Brazil (n = 50).....	92
Table 3.4- Principal Component Analysis (PCA) of soil fertility attributes, particle-size distribution, and trace element concentrations in study areas within the cacao-growing region of Bahia, northeastern Brazil.....	96
Table 3.5 - Individual Ecological Risk Index (ER <sub>i</sub> ) of trace elements in soils under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia.....	104

## LIST OF ABBREVIATIONS, INITIALS AND SYMBOLS

AFS-C	Agroforestry system of cacao
CONAMA	Brazilian National Environmental Council
CF	Contamination Factor
CRB	Cacao Region of Bahia
EF	Enrichment Factor
EPA	Environmental Protection Agency
ERi	Individual Ecological Risk
ICP – OES	Inductively coupled plasma - optical emission spectrometry
NF	Native Forest
PERI	Potential Ecological Risk
QRV	Quality Reference Values
SRM	Standard Reference Materials
TEs	Trace-elements
USEPA	United States Environmental Protection Agency

## SUMMARY

1 GENERAL INTRODUCTION .....	17
2 BIBLIOGRAPHIC REFERENCES .....	19
3 ARTICLE I: Carbon stock potential in agroforestry systems with cacao – cabruca as a climate change mitigation strategy in the Atlantic Forest of Northeast Brazil.....	21
ABSTRACT .....	23
3.1 INTRODUCION .....	24
3.2 MATERIAL AND METHODS.....	26
3.2.1 Field of study .....	26
3.2.2 Experimental arrangement.....	28
3.2.3 Litter and soil sampling.....	29
3.2.4 Total organic Carbon and Nitrogen.....	30
3.2.5 Statistical analysis.....	32
3.3 RESULTS AND DISCUSSION.....	32
3.3.1 Soil fertility and granulometry.....	32
3.3.2 Vertical distribution of carbon and nitrogen in the soil.....	35
3.3.3 Variables influencing soil carbon.....	40
3.3.4 Linear interactions between TOC and NT along the soil profile.....	41
3.3.5 Main components - PCA of chemical attributes and soil granulometry in AFS- C and NF.....	43
3.4.CONCLUSION.....	46
3.5 REFERENCES .....	47
4 ARTICLE II: Geochemical background of some trace elements in Atlantic Forest soils in a cacao-producing region and its implications for human health and food safety.....	53
ABSTRACT .....	55
4.1 INTRODUCTION.....	56
4.2 MATERIALS AND METHODS.....	58
4.2.1 Study area .....	58
4.2.2 Soil sampling and laboratory analysis.....	60
4.2.3 Trace elements and analytical quality control.....	60
4.2.3 Data set processing and statistical analysis.....	61
4.3 RESULTS AND DISCUSSION.....	61
4.3.1 Analysis quality assurance.....	61
4.3.2 Soil particles sizes and chemical characteristics.....	62
4.3.3 TEs natural concentrations.....	64
4.3.4 Correlation between soil characteristics and TEs concentration.....	66
4.3.5 Quality reference values for TEs.....	70
4.4 CONCLUSION.....	71
4.5 REFERENCES.....	72
5 ARTICLE III: Trace-element concentrations and ecological risk potential in soils under “cabruca” agroforestry system in the cacao-growing region of Bahia, northeastern Brazil .....	80
ABSTRACT .....	83
5.1 INTRODUCTION.....	84

5.2 MATERIAL AND METHODS.....	84
5.2.1 Study area.....	84
5.2.2 Experimental design, sampling, and soil analysis.....	85
5.2.3 Chemical and particle size analysis.....	86
5.2.4 Determination of trace elements in soil.....	87
5.2.5 Ecological Risk Indicators.....	87
5.2.5.1 Geoaccumulation index .....	87
5.2.5.2 Contamination factor .....	88
5.2.5.3 Enrichment factor .....	88
5.2.5.4 Individual ecological index .....	89
5.2.5.5 Potential ecological risk index .....	89
5.2.6 Statistical analysis.....	90
5.3 RESULTS AND DISCUSSION.....	91
5.3.1 Soil attributes .....	91
5.3.2 Trace element concentrations in soils under cacao cultivation.....	92
5.3.3 Relationship between environmental factors and trace element.....	93
5.3.4 Principal Component Analysis.....	95
5.3.5 Vertical distribution of trace elements in soil.....	98
5.3.6 Accumulation indices for trace elements in soils.....	100
5.4 CONCLUSION .....	105
5.5 REFERENCES.....	105
6 FINAL CONSIDERATIONS.....	112
7 ANNEX.....	114

## 1 GENERAL INTRODUCTION

The increasing appreciation of agricultural soils highlights their role in ecological sustainability, climate change mitigation, the provision of ecosystem services, and food security (Dazzi et al., 2019; Hou et al., 2020; Yang et al., 2021). To this end, understanding the factors that affect soil quality, including the processes that regulate carbon, as well as the concentration and distribution of trace elements (TEs), is essential for ensuring its functionality.

In this context, management systems play a fundamental role in the soil's ability to store carbon. Among them, agroforestry systems have gained prominence for their ability to accumulate carbon, conserve biodiversity, and improve soil health (Banwart et al. 2019). In Brazil, the use of agroforestry systems grew by 67% between 2006 and 2017, occupying 10% of the country's productive area (IBGE, 2024).

Considering the need to mitigate the environmental impacts of agriculture, practices that favor the balance between production and conservation become increasingly relevant. The impact of reducing agricultural emissions through sustainable management systems should not be underestimated (Zhang et al., 2019), as the reduction of 1 Pg of soil carbon is equivalent to an atmospheric CO<sub>2</sub> enrichment of 0.47 ppmv (Pacchiarelli et al., 2022). Therefore, analysing the potential of soil carbon stocks in agroforestry systems contributes not only to understanding the impacts of land use but also to defining climate mitigation strategies.

The cacao region of Bahia stands out nationally for its extensive cacao plantation, cultivated under shade of Atlantic Forest trees (Ribeiro et al., 2009). It is currently the largest cacao producer in Brazil (IBGE, 2024), which reinforces its agricultural and ecological relevance. The region exhibits high variability in its geomorphological, pedological, topographic, and climatic characteristics (Bastos et al. 2025). This environmental complexity of the region influences the natural concentrations and distribution of trace elements in the soil, whose concentrations vary naturally according to the geochemical composition of parent rocks and pedogenetic processes (Rezapour et al., 2022).

Although trace elements (TEs) usually occur at very low concentrations in the environment (Liu et al., 2018), they exhibit high toxicity and can pose significant risks to both human health and ecosystems even at minimal levels (Wang et al., 2018). This

concern arises from their lack of essential biological function combined with pronounced toxic effects (Duan et al., 2024). Considering the potential toxicity and bioaccumulation of TEs, the development and enforcement of strict legislation for environmental monitoring are necessary (Okereafor et al., 2020).

The determination of TEs under natural conditions forms the basis for monitoring and establishing legislation compatible with soil quality standards and local realities (Souza et al., 2024; Cardoso et al., 2024). In the same vein, indices of soil pollution, enrichment, and ecological risk can be aligned with this concept, as they depend on reference elements quantified in areas without human intervention (Keshavarzi and Kumar, 2019).

Therefore, this study aimed to evaluate soil carbon stocks, as well as the distribution and concentration of trace elements at a regional scale. The specific objectives are: (1) to quantify and compare the vertical distribution of organic carbon and total soil nitrogen stocks in different land use systems, including native vegetation of the Atlantic Forest and the cacao agroforestry system "cabruca", in order to assess their long-term carbon sequestration potential; (2) to determine the natural concentrations of potentially toxic trace elements (Ba, Cd, Co, Cr, Fe, Mo, Ni, Pb, Sb, Ti, V and Zn) in soils under native vegetation of the cacao region of Bahia, establishing a regional reference for environmental monitoring and risk assessment; (3) to establish Quality Reference Values (VRQs) for trace elements in soils of the cacao-growing region of Bahia, considering the geochemical, pedogenetic, and landscape variability of the area, in line with national and international environmental guidelines; (4) to evaluate the degree and patterns of soil contamination by trace elements along a land use gradient, identifying the main anthropogenic sources of contamination through multivariate analyses.

## 2 BIBLIOGRAPHIC REFERENCES

Banwart, S. A., Nikolaidis, N. P., Zhu, Y. G., Peacock, C. L., Sparks, D. L. Soil functions: connecting the critical zone of the Earth. **Annual Review of Earth and Planetary Sciences**, 47(1), 333-359, 2019. <https://doi.org/10.1146/annurev-earth-063016-020544>

Bastos, T. R.S., Medeiros, J. C., do Nascimento, C. W. A., Cardoso, K. M., Alves, P. N., Morais, P. G. C., Boechat, C. L. Geochemical background of some trace elements in Atlantic Forest soils in a cocoa-producing region and its implications for human health and food safety. **Chemosphere**, 377, 144341, 2025. <https://doi.org/10.1016/j.chemosphere.2025.144341>

Cardoso, K.M., Boechat, C.L., Nascimento, C.W.A., Escobar, M.E.O., Silva, D.G., Lins, S.A. S., Morais, P.G.C. Watershed-scale assessment of the environmental values of potentially toxic soil elements of the Caatinga and Atlantic Forest ecotone in Brazil. **Chemosphere** 338, 139394, 2023. <https://doi.org/10.1016/j.chemosphere.2023.139394>

CONAMA - Conselho Nacional do Meio Ambiente, 2009. Resolução N. 420 de 28 de fevereiro de 2009. <https://cetesb.sp.gov.br/areas-contaminadas/wp-content/uploads/sites/17/2017/09/resolucao-conama-420-2009-gerenciamento-de-ac.pdf>. (Accessed 15 April, 2025).

Dazzi, C., Galati, A., Crescimanno, M., Papa, G. L. Pedotechnique applications in large-scale farming: Economic value, soil ecosystems services and soil security. **Catena**, 181, 104072, 2019. <https://doi.org/10.1016/j.catena.2019.104072>

Duan, J., Biswal, B. K., Balasubramanian, R. Mobility and Fate of Potentially Toxic Elements in Soil Environment. **Advanced Sustainable Systems**. v. 8, n. 5, 2024. <https://doi.org/10.1002/adsu.202300382>

Hou, D., Bolan, N.S., Tsang, D.C., Kirkham, M.B., O'connor, D. Sustainable land use and management: an interdisciplinary and systematic approach. **Total Environmental Science**, 729, 138961, 2020. <https://doi.org/10.1016/j.scitotenv.2020.138961>

Instituto Brasileiro de Geografia e Estatística – IBGE., 2024. Levantamento Sistemático da Produção Agrícola - Área plantada, área colhida e produção, por ano da safra e produto das lavouras, janeiro, 2024.

Keshavarzi, A., Kumar, V. Ecological risk assessment and source apportionment of heavy metal contamination in agricultural soils of Northeastern Iran. **International journal of environmental health research**, 29(5), 544-560, 2019. <https://doi.org/10.1080/09603123.2018.1555638>

Liu, L.; Li, W.; Song, W.; Guo, M. Remediation techniques for heavy metal-contaminated soils: principles and applicability. **Sci. Total Environ.** v.633, p.206-219, 2018. <https://doi.org/10.1016/j.scitotenv.2018.03.161>

Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T., Mavumengwana, V. **International Journal of Environmental Research and Public Health**. v. 17, n. 7, 2020. <https://doi.org/10.3390/ijerph17072204>

Pacchiarelli, A., Priori, S., Chiti, T., Silvestri, C., Cristofori, V., Carbon sequestration of hazelnut orchards in central Italy. **Agriculture, Ecosystems & Environment**, 333, 107955. 2022. <https://doi.org/10.1016/j.agee.2022.107955>

Rezapour, S., Asadzadeh, F., Nouri, A. et al. Distribution, distribution of sources and risk analysis of heavy metals in fluvial sediments of the Lake Urmia basin. **Sci Rep**. 12, 17455, 2022. <https://doi.org/10.1038/s41598-022-21752-w>

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., Hirota, M. M. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. **Biological Conservation**, 142, 1141-1153. 2009. <https://doi.org/10.1016/j.biocon.2009.02.021>

Souza, R. E., Fontes, M. P. F., Tucci, C. A. F., Lima, H. N., Ferreira, M. S. Health risk assessment and quality reference values of potentially toxic elements in soils of the Southwestern Amazonas State – Brazil. **Science of The Total Environment**. v. 912, 2024. <https://doi.org/10.1016/j.scitotenv.2023.168937>

Wang, L.; Chen, L.; Tsang, D. C. W.; Li, J. S.; Baek, K.; Hou, D.; Ding, S.; Poon, C. S. Recycling dredged sediment into fill materials, partition blocks, and paving blocks: technical and economic assessment. **J. Clean. Prod.**, v.199, p.69-76, 2018. <http://doi.org/10.1016/j.jclepro.2018.07.165>

Yang, S., Zhao, W., Liu, Y., Cherubini, F., Fu, B.,Pereira, P. Prioritizing sustainable development goals and linking them to ecosystem services: an assessment of a global expert's knowledge. **Geography and Sustainability**, 1(4), 321–330, 2020. <https://doi.org/10.1016/j.geosus.2020.09.004>

Zhang, L.; Pang, J.; Chen, X.; Lu, Z. Carbon emissions, energy consumption and economic growth: Evidence from the agricultural sector of China's main grain-producing areas. **Science of The Total Environment**. Vol. 665, 2019, Pages 1017- 1025. <https://doi.org/10.1016/j.scitotenv.2019.02.162>

**ARTICLE I**

Carbon stock potential in agroforestry systems with cacao – cabruca as a climate change mitigation strategy in the Atlantic Forest of Northeast Brazil\*

---

\* **Situation:** Submitted

## **Carbon stock potential in agroforestry systems with cacao – cabruca as a climate change mitigation strategy in the Atlantic Forest of Northeast Brazil**

Tatiana Reis dos Santos Bastos <sup>a</sup>, João Carlos Medeiros <sup>b</sup>, Patrícia Anjos Bittencourt Barreto-Garcia <sup>a</sup>, Aldair de Souza Medeiros <sup>c</sup>, Ivan Pereira Santos Silva <sup>b</sup>, Paula Nascimento Alves <sup>c</sup>, Pâmalla Graziely Carvalho Morais <sup>c</sup>, Jose Lucas Safanelli <sup>d</sup>, Jonathan Sanderman <sup>d</sup>, Cácio Luiz Boechat <sup>a, c, \*</sup>

<sup>a</sup> State University of Southwest Bahia (UESB), Graduate Program in Agronomy, Vitória da Conquista, Bahia, 45083-900, Brazil. 2021s0001@uesb.edu.br,

<sup>b</sup> Federal University of Southern Bahia (UFSB), Itabuna, Bahia, 45600-923, Brazil. joao.medeiros@ufsb.edu.br, ivan.p.s.silva@hotmail.com

<sup>c</sup> Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas, Rodovia Bom Jesus - Viana, s/n, Planalto Horizonte, Bom Jesus, Piauí, 64900-000, Brazil.

<sup>d</sup> Woodwell Climate Research Center, 149 Woods Hole Road, Falmouth, Massachusetts, 02540, USA.

\* Corresponding author at: Campus Prof<sup>a</sup> Cinobelina Elvas, Federal University of Piauí, Rodovia Bom Jesus-Viana, km 01, S/N, Planalto Horizonte, Bom Jesus, Piauí 64900-000, Brazil.

E-mail address: cacioboecat@ufpi.edu.br (C.L. Boechat). Cellphone: +55 89 981334699

### **Highlights:**

Carbon stocks up to 60 cm highlight the carbon sink potential of the agroforestry system. Total nitrogen, litter accumulation, and the C/N ratio key drivers of soil carbon stock. Similar carbon stocks were observed between the agroforestry system and the native forest.

The "cabruca" system is an excellent option for climate change adaptation and mitigation.

## ABSTRACT

With the intensification of climate change, agricultural management practices are increasingly focused on stabilizing soil carbon stocks, which are essential for climate change mitigation. In this context, agroforestry systems have stood out as an effective management practice to cope with various climate stresses, contributing to the reduction of greenhouse gas emissions from agriculture. Therefore, the aim of this study was to evaluate how soil carbon stocks respond to the cacao agroforestry system known as “cabruca” (AFS-C), located within the Atlantic Forest biome, by analysing its maintenance and distribution along the soil profile. The study area covers the cacao-growing region of the state of Bahia (CRB), located in northeastern Brazil. We assumed that each municipality within the CRB constitutes an experimental area, represented by a farm where the predominant cultivation system is “cabruca” with over 50 years of management, in which cacao trees are variably distributed and associated with native plants. Native forest areas (NF) were used as the reference system. Litter samples were collected using a 0.0625 m<sup>2</sup> quadrat in a stratified random sampling scheme in both AFS-C and NF systems. Undisturbed soil samples were collected at four depths (0–10, 10–20, 20–40, and 40–60 cm) using an auger-type corer, totalling 188 and 152 samples for the AFS-C and NF areas, respectively. Soil samples were analysed for fertility and texture. Subsequently, total organic carbon (TOC) and total nitrogen (TN) contents were simultaneously determined using an elemental analyzer. The results indicated that, in the AFS-C system, the average accumulated carbon stock in the 0-60 cm soil profile was 113.98 Mg ha<sup>-1</sup>. Most of the carbon was stored in the deeper layers (20–60 cm), with an average stock of 26.17 Mg ha<sup>-1</sup>. This result demonstrated that AFS-C maintains high carbon levels along the soil profile, showing a behavior similar to that observed in NF, with an average stock of 31.72 Mg ha<sup>-1</sup> (20- 60 cm). The variables that most influenced soil carbon accumulation in AFS-C were TN, cation exchange capacity, and litter biomass accumulation. These findings reinforce the relevance of cacao agroforestry systems as a sustainable land-use strategy, contributing to climate change mitigation through soil carbon retention.

**Keywords:** Sustainable agriculture, Agroforestry, Native Forest, Soil climate function, Agricultural management impact.

### 3.1 INTRODUCCION

Soils have recently been incorporated into the global carbon agenda due to their vital role in climate change mitigation and adaptation (Amelung et al., 2020). Recognized as the largest terrestrial reservoir of organic carbon, soils contain approximately 2 to 2.5 times more carbon than the atmosphere (Sanderman et al., 2017). Globally, the first meter of soil stores around 1,500 Pg of carbon—about 2.7 times more than the carbon stored in plant biomass (Lal, 2018). However, anthropogenic land-use activities such as agriculture and urbanization significantly reduce soil carbon stocks and contribute to greenhouse gas (GHG) emissions (Lal, 2018). However, anthropogenic land-use activities such as agriculture and urbanization significantly reduce soil carbon stocks and contribute to greenhouse gas (GHG) emissions (Lal, 2018). However, anthropogenic land-use activities such as agriculture and urbanization significantly reduce soil carbon stocks and contribute to greenhouse gas (GHG) emissions (Lal, 2018). Moreover, GHG emissions from the agricultural sector account for 50% of CH<sub>4</sub> and 70% of N<sub>2</sub>O emissions, and estimates suggest that these gases have contributed to a cumulative global warming of 0.65 °C (Singh et al., 2022).

In 2020, the United Nations General Assembly set a goal for the international community to achieve carbon neutrality by 2050 (Zhang et al., 2024). Achieving effective carbon neutrality on a global scale requires recognizing soil organic carbon (SOC) sequestration not only as a climate change mitigation measure but also as a critical factor for soil health, soil quality, food security, and other dimensions of sustainable development (Paustian et al., 2016). In this context, the role of reducing agricultural emissions in promoting sustainable development should not be underestimated, as a 1 Pg reduction in soil carbon corresponds to an atmospheric CO<sub>2</sub> enrichment of 0.47 ppmv (Pacchiarelli et al., 2022).

In Brazil, most CO<sub>2</sub> emissions result from land-use change, deforestation, livestock production, wildfires, and agricultural activities (Brazil, 2016, 2017). In response, the country has implemented various national and international initiatives to reduce GHG emissions from land-use change (Gebara & Thuault, 2013), notably the ABC+ Plan, which aims to mitigate 1.1 billion tons of CO<sub>2</sub> by 2030 through the promotion of low-carbon agricultural practices (MAPA, 2021), including the adoption of agroforestry systems. According to the World Agroforestry (ICRAF, 2020), agroforestry integrates agriculture and forests to produce commercially valuable trees and nutritious, diverse foods while meeting global demand and addressing farmers' needs.

Agroforestry promotes biodiversity and the provision of ecosystem services (Notaro et al., 2021; Hernández-Núñez et al., 2024), such as climate regulation and

carbon sequestration (Nath et al., 2023). Data from the Brazilian Agricultural Census (2017) show that the area under agroforestry systems in Brazil increased substantially from 8.3 to 13.8 million hectares between 2006 and 2017—an increase of 67%—representing 10% of the country's productive area (IBGE, 2024).

The adoption of cacao (*Theobroma cacao* L.) agroforestry systems is considered an important alternative for the sustainable development of low-input agriculture in Brazil (Gama-Rodrigues et al., 2021), as cacao is grown alongside a rich diversity of tree species (*Schizolobium parahyba* Vell., *Erythrina poeppigiana* Walp., *Dalbergia nigra* Vell.) and other useful plants (fruits, palm trees, and ornamental plants) within the same productive area (Somarriba et al., 2013). These systems are credited with storing significant amounts of carbon in the soil (Monroe et al., 2016). However, cacao is one of the agri-food commodities whose production has doubled over the last 30 years (Parra-Paitan et al., 2023), contributing to its classification among the top 10 agricultural products at risk of driving global deforestation (Goldman et al., 2020; Pendrill et al., 2022). The current global challenge in the cacao sector is to increase production to meet growing demand without further expanding cultivation areas.

In this scenario, the cacao-growing region of Bahia stands out for its use of an agroforestry planting system based on the integration of cacao within the native understory of the Atlantic Forest, in a discontinuous and non-standardized arrangement known as the “cabruca” system (Lobão et al., 2018). According to Araújo et al. (1998), the southern region of Bahia, dominated by the Brazilian Atlantic Forest, holds the highest biodiversity indices in the country, with up to 456 tree species recorded within a single hectare, a diversity closely linked to the “cabruca” cacao system.

In Bahia, cacao cultivation covers approximately 450,000 hectares, of which around 70% is managed under the “cabruca” system within the Brazilian Atlantic Forest biome (IBGE, 2024). This highlights the importance of further investigating the role of cacao agroforestry systems as carbon sinks. Several studies have already evaluated soil carbon stocks in cacao “cabruca” agroforestry systems, especially in the cacao-growing region of Bahia (Somarriba et al., 2013; Monroe et al., 2016; Vicente et al., 2023; Faria et al., 2025), emphasizing the potential of this system in carbon conservation and climate change mitigation.

Although previous studies have contributed significantly to the knowledge about soil carbon in the cacao region, many have focused on specific areas, with limitations

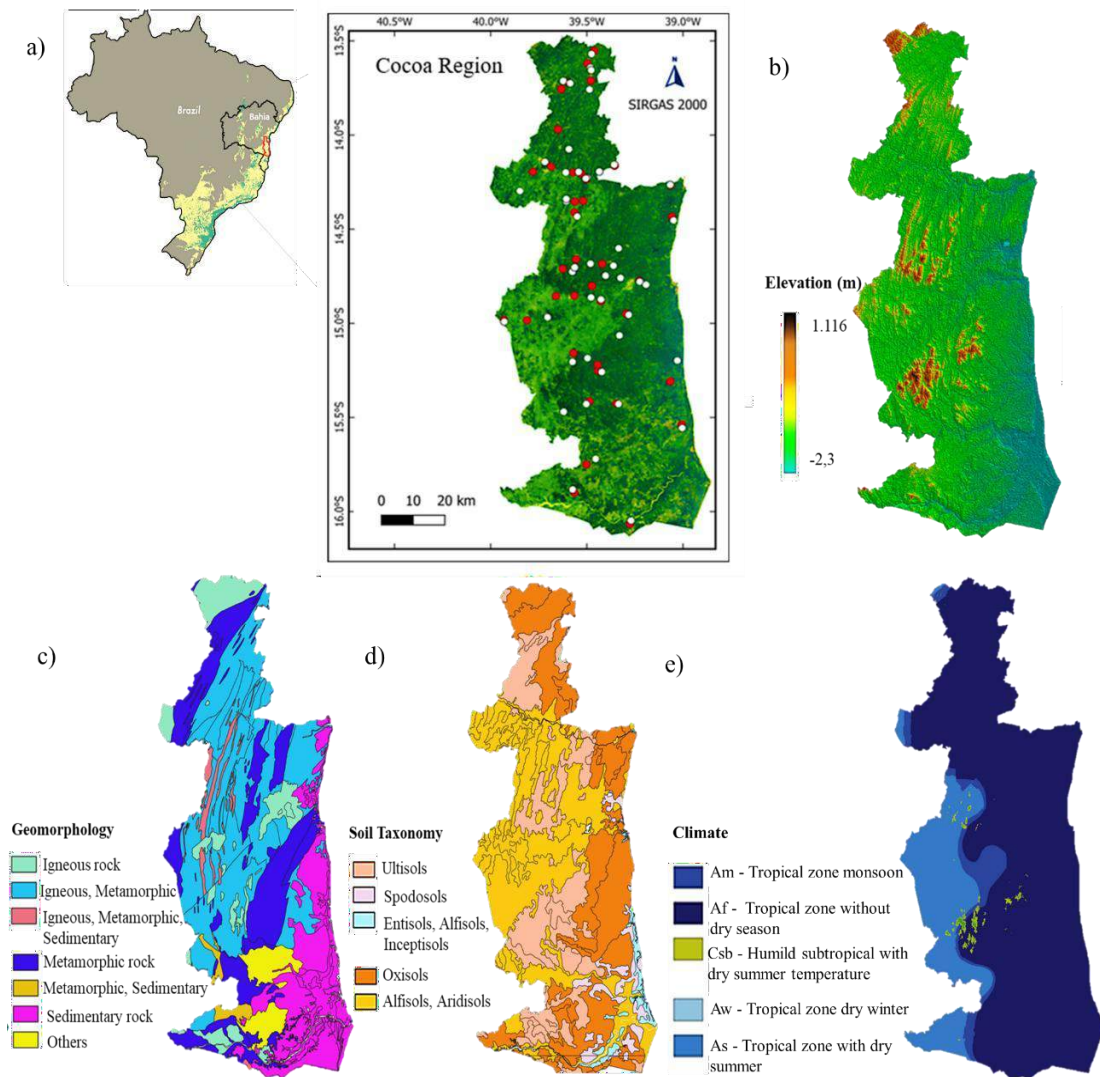
in terms of sampling intensity and spatial coverage. This work proposes to complement these findings through an unprecedented regional-scale approach, covering all municipalities in the cacao region, with the objective of quantifying and comparing the vertical distribution of organic carbon and total soil nitrogen stocks in different land use systems, including native vegetation of the Atlantic Forest and the "cabruca" cacao agroforestry system, in order to assess their long-term carbon sequestration potential.

This research is distinguished by employing a higher sampling intensity and a more homogeneous distribution of collection points throughout the study region, allowing for a more robust and comprehensive analysis of the vertical distribution and maintenance of soil carbon. Accordingly, the following hypotheses were formulated: (1) the "cabruca" cacao agroforestry system positively impacts the environment by promoting soil carbon retention; and (2) it facilitates vertical distribution of carbon throughout the soil profile.

## **3.2 MATERIAL AND METHODS**

### **3.2.1 Field of study**

Located in the Atlantic Forest biome, in northeastern Brazil, the cacao-producing region of the state of Bahia (CRB) (Fig. 1a) is a mesoregion in the southern part of the state, distinguished by the adoption of a typical cacao agroforestry system known as "cabruca", in which cacao trees grow under the shade of native forest vegetation. This mesoregion comprises 41 municipalities, located between longitudes 38°30'W and 40°00'W and latitudes 13°30'S and 16°00'S, covering an area of approximately 21,000 km<sup>2</sup>. The region has a total population of 949,916 inhabitants. The municipalities of Itabuna, with 186,708 inhabitants, and Ilhéus, with 178,703 inhabitants, play central economic roles, serving as key regional hubs for economic activities and service provision (IBGE, 2024).



**Figure 1** - Location of the cacao-growing region of Bahia (a), where the cacao agroforestry system – AFS-C and native forest – NF are established, (b) elevation, (c) geology, (d) soil classes, and (e) climate of the cacao-growing region of Bahia, northeastern Brazil.

The CRB is one of the main centers of endemism within the Brazilian Atlantic Forest, recognized for its rich biodiversity of flora and fauna (Myers et al., 2000), where cacao trees (*Theobroma cacao* L.) have found ideal conditions for development. The region adopted the “cabruca” system, a unique agricultural model that uses the canopy of Atlantic Forest trees to shelter cacao trees (Oliveira & Assis, 2023). This system has been in use for over 200 years (Lobão et al., 2011) and serves as an important mechanism for protecting the local biome. Indeed, the Atlantic Forest in southern Bahia contains some of the most well-preserved forest fragments in the state, forming part of the “Central Atlantic Forest Corridor” (Santana et al., 2020), largely due to the presence of cacao cultivation

within these fragments.

Located within the Eastern Atlantic watershed, the CRB is drained by the de Contas, Almada, and Jequitinhonha rivers. The mean annual temperature is approximately 23°C. Elevation (Fig. 1b) increases from east to west, ranging from – 2.3 m to 1,160 m above sea level. The region’s lithology (Fig. 1c) dates to the Proterozoic and Archean eons and is part of the São Francisco Craton, influenced by the Itabuna-Salvador-Curaçá orogeny. It is composed of tonalites, charnoquites, monzodiorites, and supracrustal rocks, ranging from gabbro to granite (Oliveira et al., 2010).

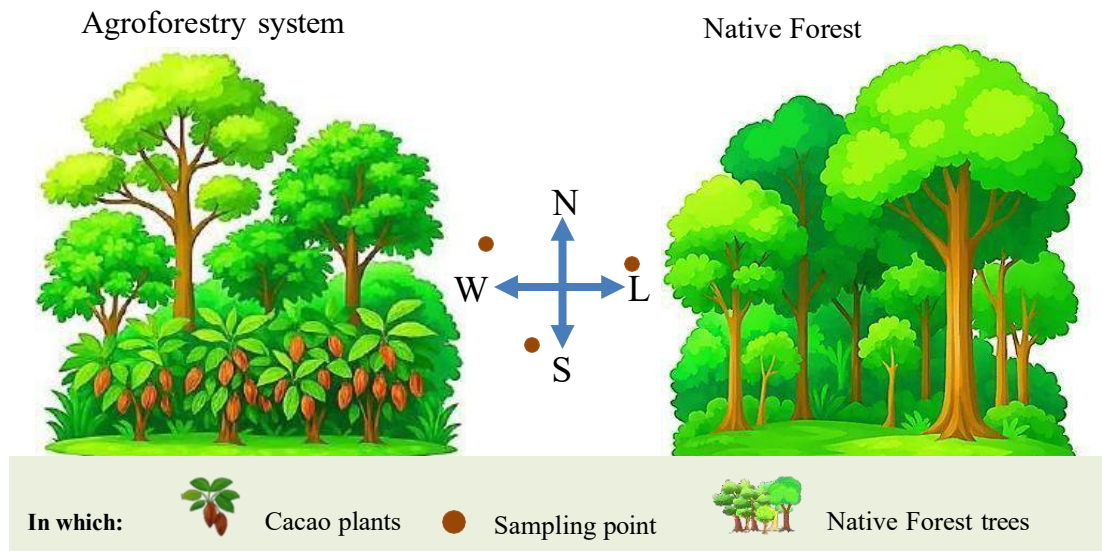
According to the U.S. Soil Taxonomy classification system (Soil Survey Staff, 2014), the main soil orders found in the region (Fig. 1d) include Oxisols, Ultisols, Alfisols, and Spodosols. Based on the Köppen-Geiger classification, the climate is predominantly tropical (Fig. 1e), including equatorial rainforest (Af), tropical savanna with dry winter (Aw), and monsoon (Am) types—hot and humid (Alvares et al., 2013). Average monthly precipitation, generally  $\geq 60$  mm, decreases from north to south and from east to west, with total annual precipitation ranging between 1,200 and 1,800 mm (Mori et al., 1983). The original vegetation of the region is predominantly Dense Ombrophilous Forest, a characteristic formation of the Atlantic Forest biome, which historically covered about 15% of Brazil’s territory (IBGE, 2012).

### 3.2.2 Experimental arrangement

In this study, each municipality within the CRB was considered an agricultural experimental unit, represented by a farm where the predominant cultivation system is “cabruca” cacao, with more than 50 years of continuous use. In recent years, all tree canopies have been replaced with clones resistant or tolerant to the fungus *Moniliophthora* (syn. *Crinipellis*) *perniciosa*, as recommended by the Executive Committee of the Cacao Planting Plan (CEPLAC, 1976). This measure has ensured the continuity of cacao production in the region despite the severe productivity crisis that occurred between 1990 and 2010. In this context, the CRB has diversified its productive areas but has maintained cacao as its primary crop. As a result, plantations are currently experiencing a rise in productivity, with regional production reaching 140,000 tons per year (IBGE, 2024).

A total of 85 sampling points were established across 41 municipalities in the

cacao-producing region, including 47 points in agroforestry system with cacao (AFS-C) areas and 38 points in native forest (NF) areas. At each experimental point, a 50 x 50 m sampling plot was randomly and independently delineated (Figure 2). The productive fields under AFS-C are exclusively managed by smallholder farmers, who do not use chemical fertilizers in their cropping systems. Their management practices include annual pruning and manual harvesting of cacao pods. The NF areas were selected near the cacao plantations and used as reference sites to represent original (pre-cultivation) conditions.



**Figure 2** - Spatial arrangement of the agroforestry system of cacao and native forest, located in the cacao-growing region of Bahia, northeastern Brazil.

### 3.2.3 Litter and soil sampling

Litter and soil samples were collected between September and December 2023, during the cacao harvest period. Accumulated litter (all plant residues on the soil surface) was collected in the same sampling plots described above, using a  $0.25 \times 0.25$  m ( $0.0625$  m<sup>2</sup>) quadrat, under a stratified random sampling design for both AFS-C and NF areas. In AFS-C plots, litter samples were taken from three positions (cacao row, shade tree row, and inter-row space), while in NF plots they were taken from three random positions. A composite sample for each plot was formed by combining the samples collected from the different positions.

After collection, the litter samples were transported to the laboratory, separated into five fractions (leaves, reproductive material, twigs, bark, and fragmented material), and oven-dried at 65 °C with forced air circulation until reaching constant weight. Accumulated litter biomass (Mg ha<sup>-1</sup>) was then calculated by extrapolating the dry weight

(g) to a per-hectare basis.

Undisturbed soil samples were collected at four depths: 0–10, 10–20, 20–40, and 40–60 cm, with three replicates per plot, using auger-type corer, totaling 188 samples in AFS-C areas and 152 in NF areas. Samples were collected from the center of each layer using 100 cm<sup>3</sup> soil cores. The three replicates from each plot were homogenized to form a composite sample, resulting in 47 AFS-C and 38 NF composite samples. Soil samples from each depth were air-dried, gently crushed, homogenized, and sieved through a 2.0 mm stainless steel mesh. Chemical analyses were carried out following the methodology of Raij et al. (2001).

Soil pH was measured in a 1:2.5 (v/v) soil-to-water suspension. Available phosphorus (P) and potassium (K) were extracted using Mehlich-1 solution (a mixture of HCl and H<sub>2</sub>SO<sub>4</sub>). Exchangeable cations—calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), and aluminum (Al<sup>3+</sup>)—were extracted with 1 mol L<sup>-1</sup> KCl and determined by titration. Potential acidity (H<sup>+</sup> + Al<sup>3+</sup>) was estimated using buffered calcium acetate solution at pH 7.0. Available micronutrients—copper (Cu<sup>2+</sup>), iron (Fe<sup>2+</sup>), manganese (Mn<sup>2+</sup>), and zinc (Zn<sup>2+</sup>)—were determined using a DTPA-TEA-CaCl<sub>2</sub> extraction solution adjusted to pH 7.3. Micronutrient concentrations were quantified by flame atomic absorption spectrometry (FAAS). Based on these results, cation exchange capacity (CEC), base saturation (V%), and aluminum saturation index (m%) were calculated as key indicators of soil fertility. Particle size analysis was performed using the pipette method, as described by Teixeira et al. (2017), which involved dispersing samples in water, agitation, controlled sedimentation, and separation of soil textural fractions using calibrated pipettes.

### **3.2.4 Total organic Carbon and Nitrogen**

For the quantification of total organic carbon (TOC) and total nitrogen (TN), soil samples were oven-dried at 105 °C for 24 hours and weighed to obtain dry mass. Bulk density (g cm<sup>-3</sup>) was calculated by dividing the oven-dried soil mass by the volume of the sampling cylinder. The analyses were performed simultaneously using elemental analysis with a Vario MAX cube elemental analyzer (Elementar Americas Inc., USA). Soil samples were homogenized, and subsamples of each <2 mm fraction were finely ground using a Retsch Mixer Mill 400 ball mill (Retsch GmbH, Germany), equipped with 50 mL hardened steel jars and one to three grinding balls, depending on the sample volume.

Grinding was conducted at a frequency of 25 Hz for 3 minutes, following the procedure described by Baldock et al. (2013).

Subsequently, the soil samples were subjected to high-temperature combustion, converting the carbon and nitrogen in the sample into CO<sub>2</sub> and N<sub>2</sub>, respectively. These gases were quantified via thermal conductivity detection, allowing for precise determination of TOC and TN concentrations. The analyses were performed without pre-treatment for carbonate removal, as the soils in this study are naturally acidic and free of carbonates. Thus, total carbon was considered equivalent to total organic carbon, and total nitrogen was determined based on the N<sub>2</sub> released during the combustion of nitrogen-containing organic matter (Nelson & Sommers, 1996; Poeplau et al., 2015).

TOC stock was calculated based on soil bulk density, sampling depth, and TOC concentration, following the approach of Nelson & Sommers (1996) and the IPCC (2006), according to Equation (1).

$$\text{TOC (Mg/ha)} = \text{BD (g/cm}^3\text{)} \times \text{C (\%)} \times \text{Z (cm)} \times 10 \quad (1)$$

Where:

TOC = Soil carbon stock, in megagrams of carbon per hectare (Mg C ha<sup>-1</sup>);

BD = Bulk density in g cm<sup>-3</sup>;

C = Soil organic carbon content in (g 100 g<sup>-1</sup>);

Z = Soil layer thickness in cm (e.g. 0–10 cm = 10);

10 = Conversion factor for Mg ha<sup>-1</sup> (since 1 hectare area = 10<sup>4</sup> m<sup>2</sup>, with appropriate unit conversion).

The ratio between total organic carbon and total nitrogen (C/N), widely used as an indicator of organic matter quality and soil biogeochemical balance, was obtained using the following Equation (2).

$$\text{C/N ratio} = \text{TOC} / \text{NT} \quad (2)$$

Where:

C/N ratio = ratio of total organic carbon to total nitrogen; TOC = total organic carbon content (g kg<sup>-1</sup>);

NT = total nitrogen content (g kg<sup>-1</sup>).

### **3.2.5 Statistical analysis**

To describe the variability of soil fertility and particle size attributes in the AFS-C and NF systems, data dispersion indices were calculated, including range, standard deviation (SD), standard error of the mean (SE), and variance. To characterize the distribution of the data, skewness and kurtosis coefficients were also computed. The entire dataset was tested for residual normality using the Shapiro–Wilk test and for homogeneity of variances using Levene’s test. Since the assumptions of normality and homoscedasticity were not met ( $p < 0.05$ ), the non-parametric Mann–Whitney test was applied to compare AFS-C and NF groups.

To estimate which soil attributes most strongly influenced TOC content in AFS-C and NF soils, the Random Forest model was applied separately for each system. Based on the model results, linear regression analysis was performed to assess the correlation between total soil carbon and the most important predictive variables ( $p < 0.05$ ). In addition, principal component analysis (PCA) was performed to reduce the multidimensional variability of the data and to visualize treatment (system) clustering based on similarities and differences across variables.

All statistical analyses were performed using the Python programming language (Python Software Foundation, 2024), version 3.11. Geographic coordinates of the soil samples were recorded using a Garmin GPS device configured with the UTM projection and Sirgas 2000 Datum (Geocentric Reference System for the Americas).

## **3.3 RESULTS AND DISCUSSION**

### **3.3.1 Soil fertility and granulometry**

The analysis of soil fertility and particle size attributes in cacao agroforestry systems (AFS-C) and native forest (NF) revealed significant variations, as shown in Table 1. Data dispersion was marked by a wide range between minimum and maximum values, particularly for P, Mn, Fe, sand, clay, and silt, indicating pronounced differences between the systems. Measures of central tendency—mean and median—were generally similar between the two systems for most variables, except for iron (Fe), which showed a substantial difference, with a variation exceeding 35%.

**Table 1.** Soil attributes of AFS-C (n = 47) and NF (n = 38), located in the cacao growing region of Bahia, northeastern Brazil.

Variable	System	Mean	Median	Min	Max	SD	SE	Skewness	Kurtosis
pH (H <sub>2</sub> O)	AFS-C	5.57	5.55	4.37	6.95	0.73	0.05	0.22	-0.94
	NF	5.66	5.62	4.35	7.94	0.75	0.06	0.20	-0.79
Al (cmolc dm <sup>-3</sup> )	AFS-C	0.42	0.0	0.0	6.25	0.85	0.06	3.36	15.59
	NF	0.54	0.0	0.0	5.0	0.85	0.06	2.27	6.54
H+Al (cmolc dm <sup>-3</sup> )	AFS-C	3.93	3.49	0.89	11.07	2.37	0.17	0.96	0.36
	NF	3.42	2.6	0.62	13.15	2.56	0.2	1.09	0.88
Ca (cmolc dm <sup>-3</sup> )	AFS-C	4.52	3.55	0.0	16.63	3.37	0.24	1.28	1.45
	NF	3.90	3.52	0.07	14.01	3.06	0.35	0.79	0.06
Mg (cmolc dm <sup>-3</sup> )	AFS-C	2.54	1.83	0.0	10.08	2.01	0.14	1.32	1.33
	NF	3.74	2.59	0.15	19.25	3.74	0.3	1.56	2.08
K (cmolc dm <sup>-3</sup> )	AFS-C	0.04	0.02	0.0	0.42	0.06	0.0	2.71	10.37
	NF	0.20	0.16	0.00	0.71	0.15	0.01	1.43	1.9
P (mg dm <sup>-3</sup> )	AFS-C	16.49	10.31	0.25	115.93	19.07	1.39	2.66	8.95
	NF	19.12	9.18	4.21	131.47	22.98	1.86	2.46	6.3
SB (cmolc dm <sup>-3</sup> )	AFS-C	7.11	5.87	0.35	24.31	4.92	0.35	1.06	0.61
	NF	8.12	6.5	0.27	46.71	6.98	0.56	1.63	5.15
CEC (cmolc dm <sup>-3</sup> )	AFS-C	11.05	9.93	2.63	27.86	9.02	0.38	1.06	0.73
	NF	11.54	9.98	0.95	47.35	7.17	0.58	1.37	3.33
V (%)	AFS-C	60.59	64.28	9.83	92.91	21.75	1.58	-0.55	-0.68
	NF	62.91	72.34	11.79	98.65	26.62	2.15	-0.49	-1.19
m (%)	AFS-C	8.71	0.0	0.0	75.63	15.98	1.16	1.98	3.14
	NF	15.68	0.0	0.0	88.94	23.76	1.92	1.36	0.55
Cu (mg dm <sup>-3</sup> )	AFS-C	3.57	2.2	0.13	32.27	4.56	0.33	3.74	17.04
	NF	2.88	1.59	0.0	17.1	3.16	0.25	1.76	3.8
Mn (mg dm <sup>-3</sup> )	AFS-C	283.11	246.94	0.10	2056.75	284.73	20.76	2.78	13.69
	NF	196.31	144.6	0.21	1919.3	277.17	22.48	1.93	4.38
Fe (mg dm <sup>-3</sup> )	AFS-C	611.12	460.93	47.3	2496.51	491.81	35.86	1.54	2.63
	NF	549.13	479.2	12.17	1930.1	414.29	33.6	0.87	0.33
Zn (mg dm <sup>-3</sup> )	AFS-C	7.35	3.92	0.42	65.43	9.33	0.68	2.98	12.22
	NF	6.52	4.16	0.09	37.28	6.76	0.54	3.75	18.53
sand (g kg <sup>-1</sup> )	AFS-C	499.43	475.64	115.92	797.93	169.44	12.35	-0.06	-0.93
	NF	492.21	487.27	179.91	878.77	169.54	13.75	0.26	-0.65
clay (g kg <sup>-1</sup> )	AFS-C	297.76	282.11	100.59	798.98	142.97	10.42	0.64	-0.18
	NF	324.07	286.41	82.14	684.2	150.96	12.24	0.58	-0.67
silt (g kg <sup>-1</sup> )	AFS-C	202.79	181.88	3.99	665.83	120.16	8.76	0.99	0.87
	NF	183.71	163.1	10.34	576.47	116.07	9.41	1.18	1.59
BD (g cm <sup>-3</sup> )	AFS-C	1.43	1.42	0.94	1.97	0.22	0.01	0.34	-0.28
	NF	1.28	1.25	0.94	1.9	0.15	0.01	0.63	2.3
TOC (g kg <sup>-1</sup> )	AFS-C	12.93	10.7	2.3	67.1	9.04	0.65	2.32	8.76
	NF	16.54	15.55	0.7	50.70	8.33	0.67	0.77	0.86
TN (g kg <sup>-1</sup> )	AFS-C	1.23	1.10	0.2	4.4	0.73	0.00	1.20	1.91
	NF	1.76	1.6	0.1	4.6	0.85	0.06	0.89	0.97

n = number of samples; H+Al = potential acidity; SB = sum of base; CEC = cation exchange capacity (at pH 7); V = base saturation; m = aluminium saturation; BD = bulk density; SD = standard deviation; SE = standard error.

A standard deviation below 1 indicates minimal data dispersion around the mean, highlighting the uniformity among samples for pH (0.73; 0.75), Al (0.85; 0.85), K (0.06; 0.15), BD (0.22; 0.15) and total nitrogen – TN (0.73; 0.85) in AFS-C and NF, respectively. However, despite the low dispersion in most variables, extreme values were observed for Mn (284.73) and Fe (491.81) in AFS-C, and Mn (277.17) and Fe (414.29) in NF. These results, derived from natural data, reinforce that fitting to a theoretical distribution is merely an approximation, as suggested by Warrick and Nielsen (1980).

The standard error of the mean (SE) showed a wide range of variation in both systems. In AFS-C, SE ranged from 0.0 to 35.86, while in NF the lowest SE was observed for BD (0.1) and the highest for Fe (33.60). This high variability reflects not only the natural dispersion and heterogeneity of the analyzed samples (Field, 2018; 2024) but also the inherent differences among variables, which is expected in multivariate datasets. The skewness of variables in AFS-C and NF revealed positively skewed (right-skewed) distributions in 91% of variables for AFS-C and 96% for NF, with values ranging from near-symmetric distributions, such as pH (0.22) and BD (0.34), to highly skewed distributions, such as Al (3.36) and Cu (3.74) in AFS-C, and pH (0.20) and sand (0.26) in NF. As highlighted by Hair et al. (2010), positive skewness values indicate a concentration of data below the mean and a higher frequency of extreme high values—typical of environmental data.

The kurtosis analysis complemented the assessment of data dispersion. Most variables diverged from the mesokurtic normal distribution (kurtosis = 0), as defined by Tabachnick and Fidell (2019). Some variables in both AFS-C and NF showed negative kurtosis, indicating platykurtic distributions—flatter curves with lighter tails—such as pH (-0.94; -0.79), V (-0.68; -1.19), sand (-0.93; -0.65), clay (-0.18; -0.67), and BD (-0.28), respectively, in AFS-C and NF.

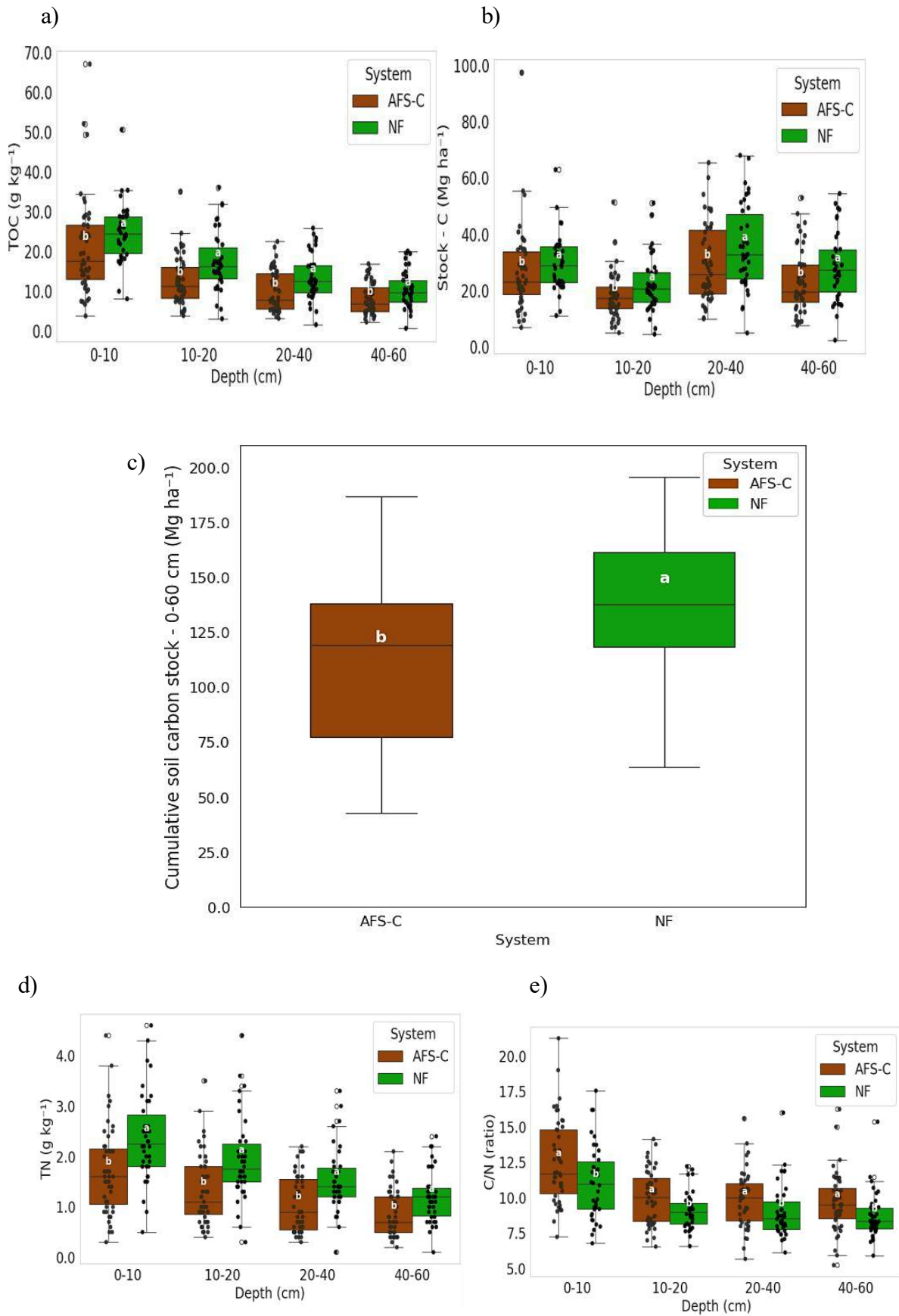
In AFS-C, several variables exhibited slightly positive kurtosis, differing from those in NF, and showed moderately leptokurtic distributions for H+Al (0.36), SB (0.61), CEC (0.73), Mg (1.33), Ca (1.45), and TN (1.91), suggesting slightly heavier tails than a normal distribution. Strongly to extremely leptokurtic curves were also identified in AFS-C for m (3.14), Fe (2.63), TOC (8.76), P(8.95), K (10.37), Zn (12.22), Mn (13.69), Al (15.59), and Cu (17.04). In NF, moderately leptokurtic distributions were observed in

H+Al (0.88), Ca (0.06), Mg (2.08), K (1.90), m (0.55), Fe (0.33), silt (1.59), BD (2.30), TOC (0.86), and TN (0.97). In contrast, NF also exhibited highly to extremely leptokurtic distributions for Al (6.54), P (6.30), SB (5.15), CEC (3.33), Cu (3.80), Mn (4.38), and Zn (18.53).

These leptokurtic results for AFS-C and NF indicate the presence of frequent extreme values associated with heavier tails compared to the normal distribution, a pattern consistent with the characteristics of the environmental data (Hair et al., 2010).

### **3.3.2 Vertical distribution of carbon and nitrogen in the soil.**

In the AFS-C system, TOC content at the 0–10 cm depth was 21.03 g kg<sup>-1</sup> ( $p = 0.009$ ), significantly higher than at 10–20 cm (12.74 g kg<sup>-1</sup>;  $p = 0.001$ ). This difference persisted in the deeper layers, where TOC contents gradually decreased along the soil profile (20–40 cm: 9.94 g kg<sup>-1</sup>,  $p = 0.002$ ; 40–60 cm: 8.04 g kg<sup>-1</sup>,  $p = 0.009$ ). In the NF system, TOC at 0–10 cm depth was considerably higher than in AFS-C, with a mean of 24.78 g kg<sup>-1</sup> (Fig. 3a).



**Figure 3** - Differences between (a) total organic carbon – TOC per depth and (b) soil carbon Stock-C, (c) cumulative soil carbon stock, (d) nitrogen – TN per depth and (e)

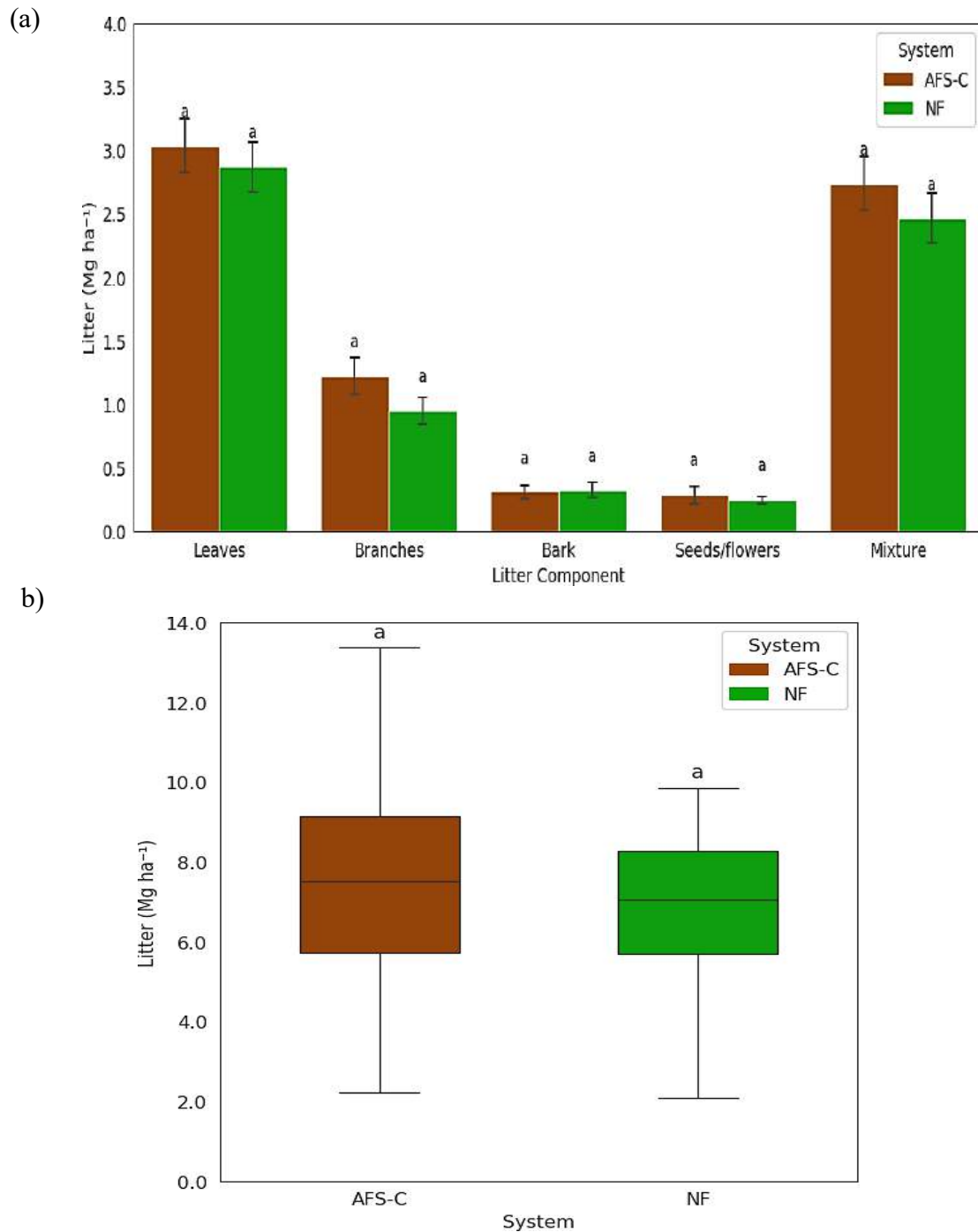
carbon-nitrogen ratio – C/N per depth in agroforestry system of cacao - AFS-C and native forest – NF, located in the cacao-growing region of Bahia, northeastern Brazil. Error bars represent the standard error of the mean. Different letters indicate significant differences between the groups, using Mann-Whitney ( $p < 0.05$ ).

The higher TOC concentrations in NF soils can be attributed to reduced soil disturbance, greater structural complexity, higher plant species diversity, and larger above-and below-ground biomass in native forest environments, which support continuous organic matter input.

The high litter accumulation observed in the NF system supports this explanation, averaging  $6.5 \text{ Mg ha}^{-1}$  and composed of bark, branches, and dry leaves from woody species (Fig. 4a, b). Due to their composition, these residues are likely rich in recalcitrant compounds such as lignin and polyphenols (Marconi & Armengot, 2020).

This trend of higher TOC in NF persisted in the deeper layers (10–20, 20–40, and 40–60 cm), with average values of 17.45, 13.54, and  $10.43 \text{ g kg}^{-1}$ , respectively. Compared to NF, TOC in AFS-C was 36.8%, 36.2%, and 29.7% lower at those same depths, maintaining the pattern of reduced carbon contents along the soil profile (Fig. 3a).

The lower level of soil disturbance in NF favors organic matter retention, resulting in higher carbon concentrations. Nevertheless, the AFS-C system still exhibited relevant TOC values (Fig. 3a), with a mean litter biomass of  $7.8 \text{ Mg ha}^{-1}$  (Fig. 4b), demonstrating its strong potential for soil carbon restoration. This is primarily due to the continuous biomass input from senescent cacao leaves, averaging  $3.1 \text{ Mg ha}^{-1}$ , and partially decomposed material (mixed litter), averaging  $2.7 \text{ Mg ha}^{-1}$  in this system (Fig. 4a). In this regard, as noted by Nair et al. (2021) and Sales et al. (2025), agroforestry systems tend to replicate the dynamics of natural ecosystems, both spatially and temporally.



**Figure 4** - Fractions of litter (a) from AFS-C and NF (b), total litter AFS-C and NF, located in the cacao-growing region of Bahia, northeastern Brazil.

Soil carbon stock (Stock-C) varied non-linearly with depth: 26.64 Mg C ha<sup>-1</sup> at the 0–10 cm layer and 17.98 Mg C ha<sup>-1</sup> at 10–20 cm layer. This result is consistent with previous studies (Monroe et al., 2016; Faria et al., 2025), which demonstrated that in “cabruca” cacao agroforestry systems in southern Bahia, the highest carbon stocks were observed in the surface layers. Compared to NF, carbon stocks in AFS-C were 11.07%

and 21.13% lower at the 0–10 and 10–20 cm depths, respectively. These findings are in line with Bremer et al. (2025), who emphasize that although agroforestry systems do not reach the carbon stock levels of native forests, they still play an important role in mitigating carbon losses. The continuous input of litter (Fig.4a, b) may contribute to increasing soil carbon stocks over time.

As depth increased, the differences between AFS-C and NF became more pronounced. The agroforestry system showed a reduction of 20.71% and 21.83% in C stock relative to NF at 20–40 cm and 40–60 cm, respectively. Nevertheless, these values still represent significant amounts of carbon in the deeper layers, with 35.27 Mg C ha<sup>-1</sup> at 20–40 cm and 28.18 Mg C ha<sup>-1</sup> at 40–60 cm.

The higher carbon concentrations in the deeper layers (20–40 cm and 40–60 cm) may be related to the presence of more lignified roots and the greater physical protection of organic matter (Liu et al., 2022). Additionally, past land use may have influenced carbon redistribution throughout the soil profile via organic residue deposition (Don et al., 2011). This pattern suggests that while the surface layer contains the most active and recent organic matter, subsurface and deep layers also play a vital role in carbon storage, particularly by retaining more stable forms with longer residence times in the soil (Rumpel & Kögel-Knabner, 2011).

When analysing the accumulated carbon stock in the soil at a depth of 0–60 cm (Fig. 3c), the AFS-C system (113.98 Mg C ha<sup>-1</sup>) showed a 23.53% reduction compared to NF (140.80 Mg C ha<sup>-1</sup>). These results are consistent with the findings of Menezes et al. (2017), who evaluated areas of the Atlantic Forest at different successional stages, including secondary forest in early stage (SFIS, ~20 years of regeneration), intermediate stage (SFINS, ~25 years), advanced stage (SFAS, ~60 years) and a mixed managed pasture (MMP). Total soil carbon stocks, measured up to 60 cm depth, showed an increasing trend across these areas: MMP (76.35 Mg C ha<sup>-1</sup>) < SFAS (80.74 Mg C ha<sup>-1</sup>) < SFINS (92.62 Mg C ha<sup>-1</sup>) < SFIS (97.60 Mg C ha<sup>-1</sup>). However, when compared with the stocks observed in the cacao-“cabruca” agroforestry system (AFS- C, ~50 years; 113.98 Mg C ha<sup>-1</sup>), the expressive capacity of this management system to accumulate carbon along the soil profile is evidenced. This finding reinforces the importance of agroforestry systems as an effective strategy for mitigating global climate change.

Significant reductions in total nitrogen (TN) were observed in the AFS-C system compared to NF, with decreases of 41.2%, 46.2%, 50.0%, and 33.3% at the 0–10, 10–20, 20–40, and 40–60 cm depths, respectively (Fig.3d). The higher TN content in NF is

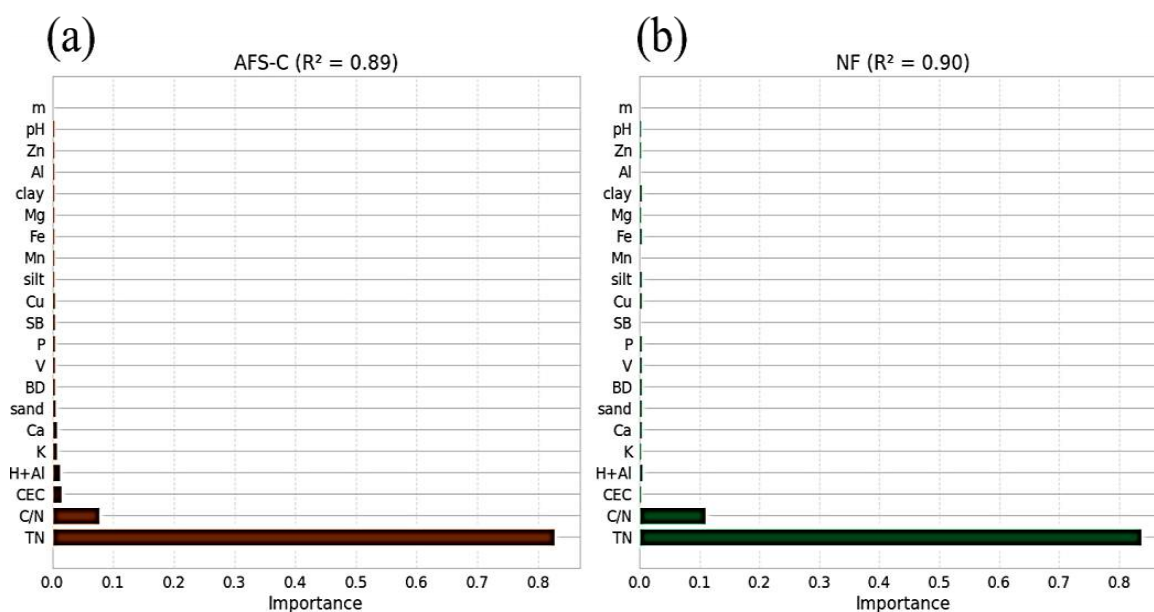
attributed to the greater variety of plant residues present, which increases nitrogen inputs to the soil.

The C/N ratio followed a similar variation pattern in both AFS-C and NF, with the highest value observed in the surface layer (0–10 cm: AFS-C = 12.47), followed by decreasing values at greater depths (AFS-C: 10.05, 9.87, 9.67, respectively). However, NF consistently exhibited lower C/N ratios across all layers (0–10 cm = 11.06; 10–20 cm = 9.10; 20–40 cm = 9.04; 40–60 cm = 8.72). The higher C/N ratio in AFS-C suggests greater litter accumulation (Fig. 4b), rich in carbon but relatively poor in available nitrogen (Fig. 3e), indicating either early-stage decomposition or slower decomposition processes due to the quality of the litter material and environmental conditions (Barbosa et al., 2017).

### **3.3.3 Variables influencing soil carbon**

Soil TOC content was influenced by both chemical properties and soil texture. According to the Random Forest model (Fig. 5a, b), total nitrogen (TN) was the most important variable explaining TOC in both systems, AFS-C ( $R^2 = 0.89$ ) and NF ( $R^2 = 0.90$ ). Since the global carbon (C) cycle is strongly coupled with nitrogen (N), it is reasonable to assume that C cycling is affected by reactive N deposition (Vitousek, 1994). Increased N deposition generally enhances SOC storage, as higher N availability can stimulate greater plant biomass production, thereby increasing C inputs into the soil (Ye et al., 2018; Wan et al., 2021).

Another influential factor for TOC in both areas, AFS-C and NF, was the C/N ratio. In AFS-C, native trees are integrated with agricultural crops, while NF exhibits high plant diversity. Consequently, the variability in the chemical composition of litter in both systems tends to promote a more balanced C/N ratio. This balance supports microbial activity, which in turn can enhance carbon accumulation in the soil (Spohn et al., 2023).

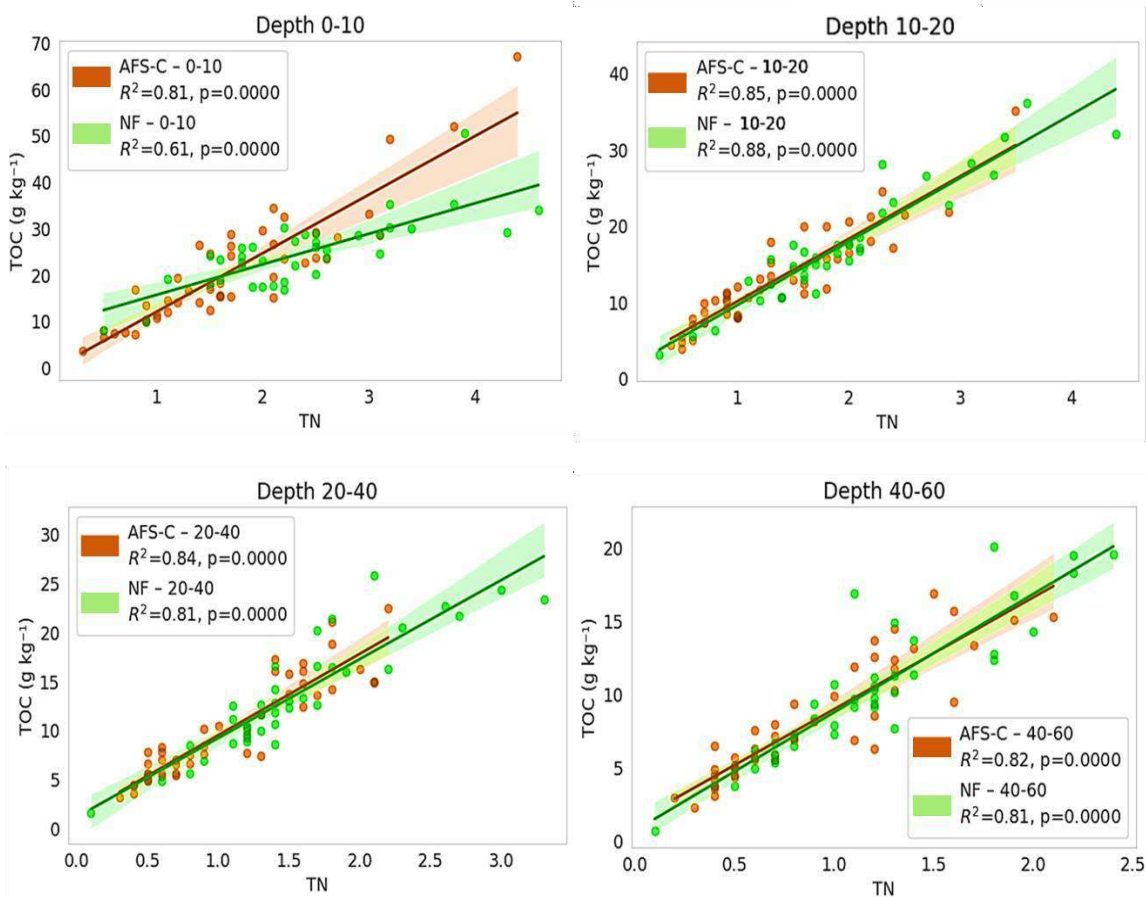


**Figure 5** - Analysis of Random Forest model showing the importance of soil fertility and granulometry attributes in explaining total soil carbon (TOC) for (a) AFS-C and (b) NF, located in the cacao-growing region of Bahia, northeastern Brazil. Soil attributes considered: m (aluminum saturation), pH, Mg, Silt, C/N (carbon/nitrogen ratio), V (base saturation), Zn, Al, Cu, Mn, SB (sum of bases), sand, P, clay, BD (bulk density), Fe, H+Al (potential acidity), K, Ca, CEC (cation exchange capacity), and TN (total nitrogen).

The high importance of CEC in explaining soil TOC in the AFS-C system (Table 1, Fig. 5a) indicates that, in this system, organic matter contributes more significantly to charge generation and, consequently, to the soil's cation exchange capacity. According to Bortoluzzi et al. (2009), organic matter accounts for approximately 75% to 90% of the CEC in tropical soils, serving as the main source of negative charges responsible for nutrient retention and exchange. As a major component of organic matter, TOC is directly linked to this function, influencing soil fertility and the ability to retain and supply essential cations.

### 3.3.4 Linear interactions between TOC and NT along the soil profile

The relationship between TOC and TN throughout the soil profile revealed a strong interdependence between these two elements (Figure 6a). At the 0–10 cm depth, the linear regression indicated that TN explained TOC variability, with  $R^2 = 0.81$  in the AFS-C and  $R^2 = 0.61$  in the NF ( $p < 0.0001$  for both systems). At this depth, the AFS-C system showed a 32.8% stronger association between TOC and TN compared to NF.



**Figure 6** - Linear regression analyses showing the relationships between total soil carbon (TOC) and TN in AFS-C and NF, in the soil layers:(a) 0-10 cm, (b) 10-20 cm, (c) 20-40 cm and (d) 40-60 cm, located in the cacao-growing region of Bahia, northeastern Brazil.

This result suggests that the surface horizon, which is more responsive to recent litter inputs (Fig. 4a, 4b), directly reflects the organic matter input promoted by the AFS-C system, owing to the steady influx of senescent material and leaf fall from cacao trees and associated species. In contrast, in NF, the greater plant diversity and heterogeneity of accumulated organic matter may have contributed to increased variability in the C-N relationship, resulting in a statistically weaker correlation.

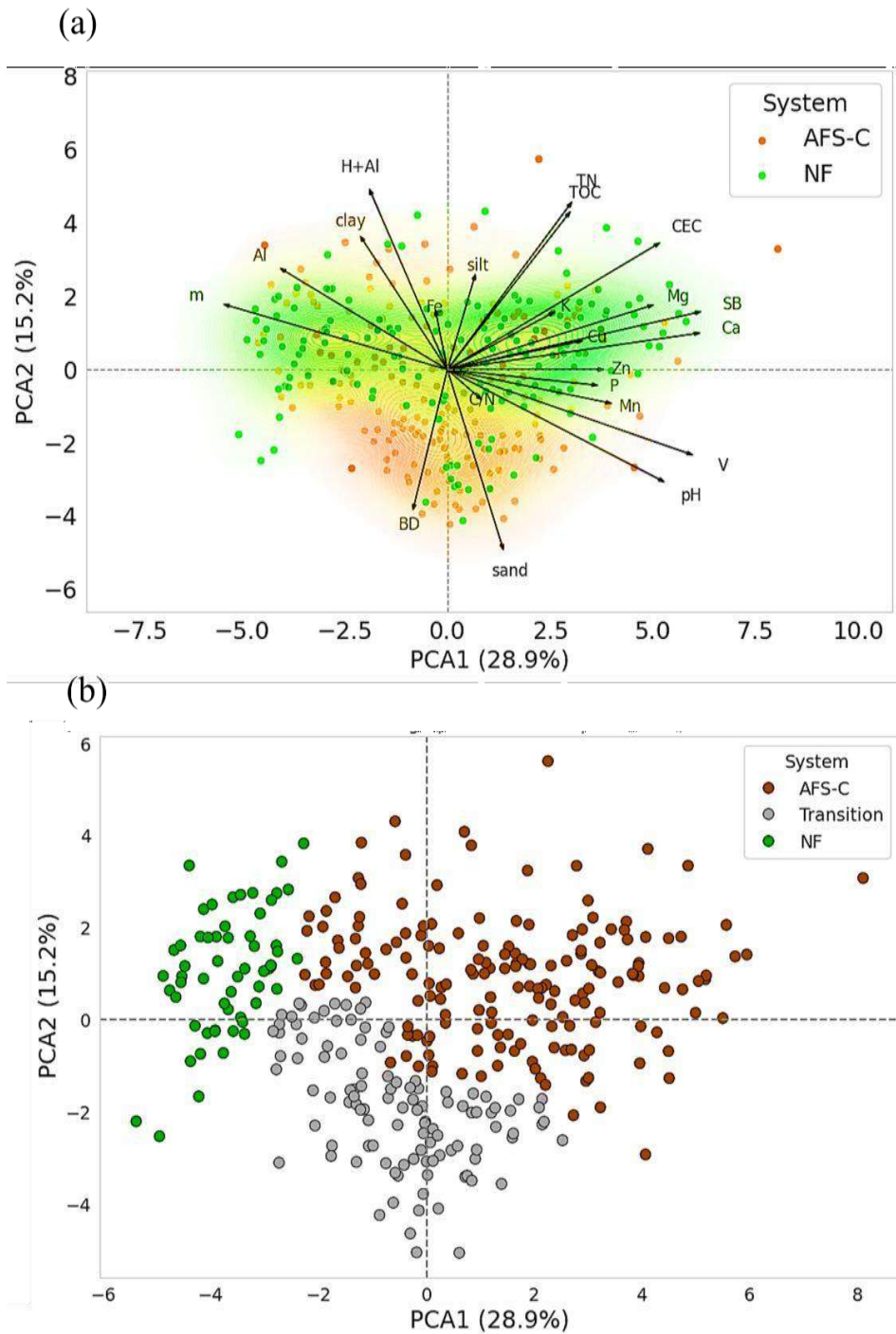
At the 10–20 cm soil layer (Fig. 6b), the observed values for TOC and TN showed little variation between systems, indicating a similar pattern in both AFS-C ( $R^2 = 0.85$ ,  $p < 0.0001$ ) and NF ( $R^2 = 0.88$ ,  $p < 0.0001$ ). This demonstrates the continued association between C and N elements throughout the soil profile. In the 20–40 cm layer (Fig. 6c), the strength of the correlation between TOC and TN remained consistent in both AFS-C ( $R^2 = 0.84$ ,  $p < 0.0001$ ) and NF ( $R^2 = 0.81$ ,  $p < 0.0001$ ). A similar trend was observed in the deepest layer (40–60 cm, Fig. 6d), with high and significant coefficients of

determination for TOC in AFS-C ( $R^2 = 0.82$ ,  $p < 0.0001$ ) and NF ( $R^2 = 0.81$ ,  $p < 0.0001$ ). The retention of C and N even in deeper layers may be attributed to the natural characteristics of soils in the cacao-growing region, which are predominantly old and highly weathered (Figure 1c and 1d), with strong pedogenetic processes that favor the formation of low-activity minerals and stable organo-mineral associations (Torn et al., 1997; Kopittke et al., 2020).

The relationship between TOC and TN in soils is well established, and management practices that promote organic matter accumulation tend to simultaneously increase soil C stocks and N availability, thereby contributing to the mitigation of greenhouse gas emissions such as CO<sub>2</sub> and N<sub>2</sub>O (Belay et al., 2025; Lal, 2015).

### **3.3.5 Main components - PCA of chemical attributes and soil granulometry in AFS- C and NF**

Principal component analysis (PCA) combined with hierarchical clustering (Ward's method) revealed a clear separation between the AFS-C and NF systems, explaining 44% of the total variance, considering the high complexity of environmental data. The first principal component (PCA1) (Fig. 7a; Table 2) accounted for 28.85% of the total variance and showed a strong contribution from the variables pH (0.73), Ca (0.85), SB (0.86), and CEC (0.72), indicating a significant association among soil fertility-related attributes. According to the cluster grouping, the AFS-C data points were distributed on the right side of the PCA1 axis.



**Figure 7** - Biplot from (a) principal component analysis and (b) hierarchical clustering (Ward's method) of soil fertility and granulometry attributes: m - aluminum saturation, pH, Mg, silt, C/N - carbon/nitrogen ratio, V - base saturation, Zn, Al, Cu, Mn, SB - sum of bases, sand, P, clay, BD - bulk density, Fe, H+Al - potential acidity K, Ca, CEC - cation exchange capacity, and TN - total nitrogen in AFS-C and NF, located in the cacao-growing region of Bahia, northeastern Brazil.

**Table 2.** Principal component analysis (PCA) of soil chemical and granulometric properties in agroforestry systems of cacao and native forests, located in the cacao-growing region of Bahia, northeastern Brazil

Variable	PC1	PC2
pH	0.73	-0.42
Al	-0.56	0.38
H+Al	-0.26	0.68
Ca	0.85	0.13
Mg	0.69	0.24
K	0.35	0.21
P	0.50	-0.05
SB	0.86	0.22
CEC	0.72	0.48
V	0.83	-0.32
m	-0.75	0.24
Cu	0.45	0.00
Zn	0.52	-0.00
Mn	0.55	0.21
Fe	-0.03	0.18
sand	0.18	-0.68
clay	-0.29	0.50
silt	0.09	0.35
BD	-0.11	-0.52
TOC	0.41	0.59
TN	0.42	0.63
C/N	0.10	-0.10

Variance by component	Eigenvalue	% Total variance	Cumulative variance (%)
PC1	6.36	28.85	44.0
PC2	3.34	15.15	

**Bold >7.0**

On the other hand, the variables sand, H<sup>+</sup>+Al, TN, and TOC were more strongly associated with PCA2 (15.15%), which explained a smaller yet still significant portion of the variance, representing characteristics related to soil texture, potential acidity, and organic matter availability. The NF samples clustered closer to the PCA2 axis (Fig. 7a, 7b), indicating more naturally preserved soil characteristics and lower internal variation, which reflects greater homogeneity. The transitional area, represented by the intermediate cluster (Fig. 7b), displayed characteristics between AFS-C and NF, likely corresponding to edge zones where the two systems overlapped.

A clear separation between AFS-C and NF was therefore observed. AFS-C samples were mainly associated with higher values of pH, base saturation (V), C/N ratio, bulk density (BD), and sand content, suggesting a trend toward increased soil fertility and

lower acidity. In contrast, NF samples were closely related to higher Al content, potential acidity ( $H^+ + Al$ ), clay content, and iron (Fe), reflecting more acidic and weathered soil conditions. The variables TOC and TN had a strong influence on PCA2 (Fig. 7a) and were more aligned with NF soils, likely due to the greater accumulation of plant residues. Soils with a higher clay content tend to exhibit greater capacity to stabilize organic carbon, due to the formation of organo-mineral complexes that protect organic matter from decomposition (Cai et al., 2016).

The results of this study confirm the important role of cacao-based agroforestry systems (AFS-C) in soil carbon accumulation, consistent with previous findings (Monroe et al., 2016). However, the variability observed in carbon levels throughout the soil profile, relative to native forest and among soil compartments, indicates that management practices still exert a significant influence on carbon storage capacity.

The implications of these results for sustainable land management are considerable. First, the maintenance and enhancement of soil carbon stocks are directly associated with chemical and biological fertility, leading to greater nutrient availability, particularly nitrogen. The synergy between carbon and nitrogen, as well documented by Lal (2015) and Belay et al. (2025), supports not only agricultural productivity but also ecosystem resilience to climate stressors such as prolonged drought and extreme temperature fluctuations.

Furthermore, practices that enhance soil organic matter, such as adopting more diverse tree species consortia, retaining litterfall, and implementing rational shade management, can strengthen long-term carbon sequestration, positioning AFS-C as effective climate change mitigation strategies.

### **3.4 CONCLUSION**

Previous studies have emphasized the growing need for more detailed information on soil carbon stocks throughout the profile, as well as on the variables and factors that influence carbon stability in soils. The results of this study indicate that agroforestry systems with "cabruca" cacao (SAF-C) promote greater carbon accumulation in the deeper soil layers (20–60 cm), reinforcing their potential as important carbon sinks.

The multidimensional assessment of the data revealed a clear similarity between the AFS-C and native forest (NF) systems, attributed to the positive influence of both on total carbon (TOC), total nitrogen (TN), and litter accumulation. The findings suggest that

the AFS-C system holds significant potential for enhancing soil carbon accumulation by fostering a more stable microenvironment conducive to carbon retention, functionally approaching NF in terms of carbon-related ecosystem services.

These results reinforce the relevance of “cabruca” agroforestry systems as a sustainable land-use strategy, contributing not only to environmental conservation but also to climate change mitigation through soil carbon retention. Moreover, these findings are expected to inform resource allocation, policy development, and evidence-based decision-making for environmental management through agroforestry interventions, particularly in cacao cultivation.

### 3.5 REFERENCES

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. D. M., Sparovek, G., 2013. Köppen’s climate classification map for Brazil. *Meteorologische zeitschrift*, 22(6), 711-728. <https://doi.org/10.1127/0941-2948/2013/0507>

Amelung, W., Bossio, D., de Vries, W., Kögel-Knabner, I., Lehmann, J., Amundson, R., Bol, R., Collins, C., Lal, R., Leifeld, J., Minasny, B., Pan, G., Paustian, K., Rumpel, C., Sanderman, J., van Groenigen, J. W., Mooney, S., van Wesemael, B., Wander, M., Chabbi, A., 2020. Towards a climate mitigation strategy. *Nature Communications*, 11, 5427, 2020. <https://doi.org/10.1038/s41467-020-18887-7>

Araújo, M.; Alger, K.; Mesquita, C. A. B.; Rocha, R., 1998. A Mata Atlântica do Sul da Bahia: Situação atual, ações e perspectivas. Caderno 08. Conselho Nacional da Reserva da Biosfera da Mata Atlântica, São Paulo.

Baldock, J. A., Sanderman, J., Macdonald, L. M., Puccini, A., Hawke, B., Szarvas, S., McGowan, J., 2013. Quantification of soil organic carbon allocation for biologically significant fractions. *Soil Research*, 51(8), 561-576. <https://doi.org/10.1071/SR12374>

Barbosa, V., Barreto-Garcia, P., Gama-Rodrigues, E., Paula, A. D. 2017. Biomassa, carbono e nitrogênio na serapilheira acumulada de florestas plantadas e nativa. *Floresta e Ambiente*, 24, e20150243. <https://doi.org/10.1590/2179-8087.024315>

Belay, A., Abegaz, A., Assen, M., 2025. Impacts of watershed management and topographic positions on soil organic carbon and total nitrogen stocks in Northeast highlands of Ethiopia. *Trees, Forests and People*, 19, 100750. <https://doi.org/10.1016/j.tfp.2024.100750>

Bortoluzzi, E. C., Rheinheimer, D. S., Petry, C., Kaminski, J., 2009. Contribution of soil constituents to the cation exchange capacity obtained by different extraction methods. *Revista Brasileira de Ciência do Solo*, 33, 507-515. <https://doi.org/10.1590/S0100-06832009000300004>

Brazil M. C. T. I., 2016. Third national communication of Brazil to the United Nations

framework convention on climate change. Bremer, L. L., McGuire, G., Silao, Z. H., Kurashima, N., Ticktin, T., Crow, S. E., Giardina, C. P., Winter, K. B., DeMaagd, N., Trauernicht, C., 2025. Carbon benefits through agroforestry transitions on unmanaged fallow farmland in Hawaii. *Scientific Reports*, 15, 5097. <https://doi.org/10.1038/s41598-025-87891-y>

Don, A., Schumacher, J., Freibauer, A. 2011. Impact of tropical land-use change on soil organic carbon stocks—a meta-analysis. *Global Change Biology*, 17(4), 1658-1670. <https://doi.org/10.1111/j.1365-2486.2010.02336.x>

Field, A., 2018. *Discovering statistics using IBM SPSS statistics*.

Field, A., 2024. *Discovering statistics using IBM SPSS statistics*. Sage publications limited.

Gama-Rodrigues, A. C., Müller, M. W., Gama-Rodrigues, E. F., Mendes, F. A. T., 2021. Cacao-based agroforestry systems in the Atlantic Forest and Amazon Biomes: An ecoregional analysis of land use. *Agricultural Systems*, 194, 103270. <https://doi.org/10.1016/j.agsy.2021.103270>

Gebara, M.F.; Thuault, A., 2013. GHG mitigation in the land use sector in Brazil: an introduction to the current national political scenario. Washington DC: WRI.

Goldman, E.; Weisse, M.; Harris, N.; Schneider, M., 2020. Estimate the role of seven commodities in agriculture-related deforestation: oil palm, soybeans, cattle, wood fiber, cacao, coffee, and rubber. Technical Note, World Resources Institute.

Guidelines for National Greenhouse Gas Inventories – IPCC., 2006. Vol. 4: Agriculture, Forestry and Other Land Use. Prepared by the National Greenhouse Gas Inventories Programme. IGES, Japan.

International Centre for Research in Agroforestry – ICRAF., 2020. World Agroforestry. Available at: <https://www.worldagroforestry.org/>. Acesso em: 28 fev. de 2025.

Instituto Brasileiro de Geografia e Estatística - IBGE., 2017. Censo Agropecuário. URL: <https://sidra.ibge.gov.br/pesquisa/censo-agropecuario/censoagropecuario-2017>.

Instituto Brasileiro de Geografia e Estatística – IBGE., 2024. Levantamento Sistemático da Produção Agrícola - Área plantada, área colhida e produção, por ano da safra e produto das lavouras, janeiro, 2024.

Hair Jr, J. F., Black, W. C., Babin, B. J., Anderson, R. E., 2010. Multivariate data analysis. In *Multivariate data analysis* (pp. 785-785), Pearson Prentice Hall: New Jersey.

Hernández-Nuñez, H. E., Suárez, J. C., Andrade, H. J., Acosta, J. R. S., Núñez, R. D., Gutiérrez, D. R., Casanoves, F., 2024. Interactions between climate, shaded canopy characteristics, and cacao production in Colombia. *Frontiers in Sustainable Food Systems*, 8, 1295992. <https://doi.org/10.3389/fsufs.2024.1295992>

Kopittke, P. M., Dalal, R. C., Hoeschen, C., Li, C., Menzies, N. W., Mueller, C. W., 2020. Soil organic matter is stabilized by organo-mineral associations through two key

processes: The role of the carbon to nitrogen ratio. *Geoderma*, 357, 113974. <https://doi.org/10.1016/j.geoderma.2019.113974>

Lal, R., Smith, P., Jungkunst, H. F., Mitsch, W. J., Lehmann, J., Nair, P. K. R., McBratney, A. B., Sá, J. C. M., Schneider, J., Zinn, Y. L., Skorupa, A. L. A., Zhang, H., Minasny, B., Srinivasrao, C., Ravindranath, N. H., 2018. The carbon sequestration

potential of terrestrial ecosystems. *Journal of Soil and Water Conservation*, 73(6), 145A-152A. <https://doi.org/10.2489/jswc.73.6.145A>

Laborde, D., Mamun, A., Martin, W., Piñeiro, V., Vos, R., 2021. Agricultural subsidies and global greenhouse gas emissions. *Nature Communications*, 12, 2601. <https://doi.org/10.1038/s41467-021-22703-1>

Liu, R., He, Y., Du, Z., Zhou, G., Zhou, L., Wang, X., Li, N., Yan, E., Feng, X., Liang, C., Zhou, X., 2022. Root production and carbon inputs derived from microorganisms jointly drive the rapid accumulation of carbon in the soil in the early stages of forest succession. *Forests*, 13(12), 2130. <https://doi.org/10.3390/f13122130>

Lobão, D.E., 2011. Sistema Cacau Cabruca e a Mata Atlântica: diversidade arbórea, conservação e potencial de produção. *Agrotropica*, 23(2,3), 115 – 124. Lobão, D. E., 2018. Cacau-cabruca: um modelo sustentável de agricultura tropical. Centro de Pesquisas do Cacau, Ilhéus, Bahia, Brasil. Disponível em: <[http://www.ceplac.gov.br/radar/sistema\\_agro.htm](http://www.ceplac.gov.br/radar/sistema_agro.htm)>. Acesso em: 20 fev. 2024 Marconi, L.

Armengot, L., 2020. Complex agroforestry systems against biotic homogenization: The case of plants in the herbaceous stratum of cacao production systems. *Agriculture, Ecosystems & Environment*, 287, 106664. <https://doi.org/10.1016/j.agee.2019.106664>

Menezes, C. E. G., Guareschi, R. F., Pereira, M. G., Anjos, L. H. C., Correia, M. E. F., Balieiro, F. C., & Piccolo, M. D. C. (2017). Organic matter in secondary forest and pasture areas. *CERNE*, 23(3), 283-290. <https://doi.org/10.1590/01047760201723032333>

Monroe, P. H. M, Gama-Rodrigues, E. F., Gama-Rodrigues, A. C., Marques, J. R. B., 2016. Soil carbon stocks and origin under different cacao agroforestry systems in Southern Bahia, Brazil. *Agriculture, Ecosystems & Environment*, 221, 99-108. <https://doi.org/10.1016/j.agee.2016.01.022>

Mori, S. A., Boom, B. M., Carvalho, A. M., Santos, T.S., 1983. Southern Bahian moist forests. *The Botanical Review*, 49(2), 155–232. <https://doi.org/10.1007/BF02861011>

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Fonseca, G. A. B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858. <https://doi.org/10.1038/35002501>

Nair, P. R., Kumar, B. M., & Nair, V. D., 2021. An introduction to agroforestry: four decades of scientific developments (pp. 1-649). Cham: Springer. <https://doi.org/10.1007/978-3-030-75358-0>

- Nath, A.J, Nath, P.C, Sileshi, G.W., 2023. Payment for Agroforestry Ecosystem Services: Case Studies and Lessons. In: Dagar, J. C, Gupta, S. R, Sileshi, G. W (eds) *Agroforestry for Sustainable Agricultural Intensification in Asia and Africa*. Sustainability Sciences in Asia and Africa. Springer, Singapore. [https://doi.org/10.1007/978-981-19-4602-8\\_23](https://doi.org/10.1007/978-981-19-4602-8_23)
- Nelson, D. W., Sommers, L. E., 1996. Total carbon, organic carbon, and organic matter. *Methods of soil analysis: Part 3. Chemical methods*, 5, 961-1010. <https://doi.org/10.2136/sssabookser5.3.c34>
- Notaro, M., Collado, C., Depas, J. K., Dumovil, D., Denis, A. J., Deheuvels, O., Gary, C., 2021. The spatial distribution and height of associated crops influence cacao tree productivity in complex agroforestry systems. *Agronomy for Sustainable Development*, 41(5), 60. <https://doi.org/10.1007/s13593-021-00716-w>
- Oliveira, B., Assis, P. R., 2023. Do cacau ao chocolate: uma análise dos desafios encontrados por empreendedores do ramo da agroindústria do cacau no sul da Bahia. *Revista Ibero-Americana de Humanidades, Ciências e Educação*, 9(9), 4799–4816. <https://doi.org/10.51891/rease.v9i9.11613>
- Pacchiarelli, A., Priori, S., Chiti, T., Silvestri, C., Cristofori, V., 2022. Carbon sequestration of hazelnut orchards in central Italy. *Agriculture, Ecosystems & Environment*, 333, 107955. <https://doi.org/10.1016/j.agee.2022.107955>
- Pendrill, F., Gardner, T.A., Meyfroidt, P., Persson, A, Adams, J., Azevedo, T., West, C., 2022. Unraveling the numbers behind tropical deforestation driven by agriculture. *Science*, 377(6611), eabm9267, 2022. <https://doi.org/10.1126/science.eabm9267>
- Parra-Paitan, C.; Ermgassen, E. K.; Meyfroidt, P.; Verburg, P. H., 2023. Large gaps in voluntary sustainability commitments covering the global cacao trade. *Global Environmental Change*, 81, 102696. <https://doi.org/10.1016/j.gloenvcha.2023.102696>
- Paustian, K., Lehmann, J., Ogle, S. et al., 2016. Climate-smart soil. *Nature* 532, 49–57. <https://doi.org/10.1038/nature17174>. Python Software Foundation, 2024. Python: A dynamic, open-source programming language. Version 3.11. Available at: <https://www.python.org>.
- Raij, B., Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Chemical analysis for the evaluation of the fertility of tropical soils. IAC. Rowley, M. C., Grand, S. Verrecchia, É. P., 2018. Calcium-mediated soil organic carbon stabilization. *Biogeochemistry* 137, 27–49. <https://doi.org/10.1007/s10533-017-0410-1e>
- Sales, E. P. O., Barreto-Garcia, P. A. B., Monroe, P. H. M., Pereira, M. G., da Silva Martins, K. B., dos Santos, T. O., Nunes, M. R. 2025. Do coffee agroforestry systems favor carbon and glomalin input in soil biogenic aggregates? *Catena*, 249, 108685. <https://doi.org/10.1016/j.catena.2024.108685>
- Sanderman, J.; Hengl, T.; Fiske, G. J., 2017. Soil carbon debt from 12,000 years of human land use. *Annals of the National Academy of Sciences*, 114(36), 9575-9580. <https://doi.org/10.1073/pnas.1706103114>

Santana, R.O.; Delgado, R.C.; Schiavetti, A., 2020. The past, present and future of vegetation in the Central Atlantic Forest Corridor, Brazil. *Remote Sensing Applications: Society and Environment*, v. 20, n. 1, p. 1-14, 2020. <https://doi.org/10.1016/j.rsase.2020.100357>

Singh, U., Algren, M., Schoeneberger, C., Lavallais, C., O'Connell, M. G., Oke, D., Dunn, J. B., 2022. Technological avenues and market mechanisms to accelerate methane and nitrous oxide emissions reductions. *Iscience*, 25(12). <https://doi.org/10.1016/j.isci.2022.105661>

Soil Survey Staff., 2014. *Keys to Soil Taxonomy*, twelfth ed. USDA - Natural Resources Conservation Service, Washington, DC.

Somarriba, E., Cerda, R., Orozco, L., Cifuentes, M., Dávila, H., Espin, T., Deheuvels, O., 2013. Carbon stocks and cacao yields in agroforestry systems of Central America. *Agriculture, Ecosystems & Environment*, Vol. 173, Pages 46-57. <https://doi.org/10.1016/j.agee.2013.04.013>

Spohn, M., Bagchi, S., Biederman, L.A et al., 2023. The positive effect of plant diversity on soil carbon depends on climate. *Nature Commun*, 14, 6624. <https://doi.org/10.1038/s41467-023-42340-0>

Tabachnick, B. G., Fidell, L. S., & Ullman, J. B., 2019. *Using multivariate statistics* (Vol. 5, No. 7). Boston, MA: Pearson. Teixeira, P. C., Donagemma, G. K., Fontana, A., Teixeira, W. G., 2017. *Manual de métodos de análise de solo*. 3. ed. rev. e ampl. – Brasília, DF.

Torn, M. S., Trumbore, S. E., Chadwick, O. A., Vitousek, P. M., Hendricks, D. M., 1997. Mineral control of soil organic carbon storage and turnover. *Nature*, 389, 170–173. <https://doi.org/10.1038/38260>

Ugbaje, S. U., Karunaratne, S., Bishop, T., Gregory, L., Searle, R., Coelli, K., Farrell, M., 2024. Space-time mapping of soil organic carbon stock and its local drivers: Potential for use in carbon accounting. *Geoderma*, Vol. 441, 116771. <https://doi.org/10.1016/j.geoderma.2023.11677>

Vicente, L. C, Gama-Rodrigues, E. F, Aleixo, S., Gama-Rodrigues, A. C, Andrade, G. R. P 2023. Chemical composition of organic carbon in aggregate density fractions in cacao agroforestry systems in southern Bahia, Brazil. *Journal of Soil Science and Plant Nutrition*, 1-15. <https://doi.org/10.1007/s42729-022-01083-5>

Vitousek, P. M 1994. *Beyond Global Warming: Ecology and Global Change*. *Ecology*, 75 (7), 1861–1876. <https://doi.org/10.2307/1941591>

Ye, C. L., Chen, D.M., Hall, S.J., Pan, S., Yan, XB, Bai, T.S., Guo, H., Zhang, Y., Bai, Y., Hu, S. 2018. Reconciling multiple impacts of nitrogen enrichment on soil carbon: plant, microbial, and geochemical controls. *Ecology Letters*, 21 (8), 1162–1173. <https://doi.org/10.1111/ele.13083>

Wan, D., Ma, MK, Peng, N., Luo, XS, Chen, WL, Cai, P., Wu, L., Pan, H., Chen, J., Yu, G., & Huang, Q. 2021. Effects of long-term fertilization on calcium-associated soil

organic carbon: implications for C sequestration in agricultural soils. *Total Environmental Science*, 772, 145037. <https://doi.org/10.1016/j.scitotenv.2021.145037>

Warrick, A.W.; Nielsen, D.R., 1980. Spatial variability of soil physical properties in the field. *Applications of Soil Physics*, p.319-344. <https://doi.org/10.1016/b978-0-12-348580-9.50018-3>

Zhang, L.; Pang, J.; Chen, X.; Lu, Z., 2019. Carbon emissions, energy consumption and economic growth: Evidence from the agricultural sector of China's main grain-producing areas. *Science of The Total Environment*. Vol. 665, 2019, Pages 1017-1025. <https://doi.org/10.1016/j.scitotenv.2019.02.162>

Zhang, W., Ma, L., Wang, X., Chang, X., & Zhu, Z., 2024. The impact of non-grain conversion of cultivated land on the relationship between agricultural carbon supply and demand. *Applied Geography*, 162, 103166. <https://doi.org/10.1016/j.apgeog.2023.103166>

**ARTICLE II**

Geochemical background of some trace elements in Atlantic Forest soils in a cacao-producing region and its implications for human health and food safety \*

---

\* **Situation:** Published  
(<https://doi.org/10.1016/j.chemosphere.2025.144341>)

## **Geochemical background of some trace elements in Atlantic Forest soils in a cacao- producing region and its implications for human health and food safety\***

Tatiana Reis dos Santos Bastos <sup>a</sup>, João Carlos Medeiros <sup>b</sup>, Clístenes Williams Araújo do Nascimento <sup>c</sup>, Kaíque Mesquita Cardoso <sup>a,d</sup>, Paula Nascimento Alves <sup>e</sup>, Pâmalla Graziely Carvalho Morais <sup>e</sup>, Geison dos Santos Pereira <sup>b</sup>, Ana Luísa Leite Pereira <sup>a</sup>, Maria Eugenia Ortiz Escobar <sup>f</sup>, Cácio Luiz Boechat <sup>a,e \*</sup>

<sup>a</sup> State University of Southwest Bahia (UESB), Graduate Program in Agronomy, Vitória da Conquista, Bahia, 45083-900, Brazil

<sup>b</sup> Federal University of Southern Bahia (UFSB), Itabuna, Bahia, 45600-923, Brazil

<sup>c</sup> Federal Rural University of Pernambuco (UFRPE), Recife, Pernambuco, 52171- 900, Brazil

<sup>d</sup> Federal Institute of Education, Science and Technology of Northern Minas Gerais (IFNMG), Araçuaí, Minas Gerais, 39600-000, Brazil

<sup>e</sup> Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas, Rodovia Bom Jesus

- Viana, S/n, Planalto Horizonte, Bom Jesus, Piauí, 64900-000, Brazil

<sup>f</sup> Federal University of Ceara (UFC), Fortaleza, Ceará, 60455760, Brazil

### **Highlights**

A high concentration of naturally occurring cadmium and barium was reported in certain areas.

Concentrations strongly correlate with parent material and pedogenetic processes.

Quality Reference Values in Atlantic Forest soils were proposed at the 75th percentile.

These values will serve as a basis for monitoring and enforcement in the region.

## ABSTRACT

The background values for trace elements (TEs) in soils are crucial for environmental monitoring and food safety, as establishing quality reference values (QRVs) and help distinguish natural from anthropogenic sources. Brazil is the sixth-largest cacao producer globally, with plantations concentrated in northeast and north regions. However, data on natural TE concentrations in soils remain scarce, posing a significant challenge for food safety, human health, and environmental regulations. This study aims to determine natural TE concentrations and establish QRVs for selected elements. Surface soil samples were collected from 38 native forest units and analyzed using exploratory, univariate, and multivariate statistical methods. Compared to soils around the world and from northern and northeastern Brazil, Ba and Cd ( $66.8$  and  $2.7$  mg kg<sup>-1</sup>, respectively) exhibited the highest mean concentrations, except when compared to European soils. The results highlight that TE concentrations in Atlantic Forest soils of southern Bahia differ due to geological diversity. Pedogenetic processes influenced TE distribution, following the order: Fe > Ti > Ba > V > Cr > Zn > Cu > Co > Ni > Pb > Cd > Sb > Mo. QRVs were established using the 75th and 90th percentiles. Future research could integrate ecological and human health risk indices in cacao-cultivated areas, considering soil, litter, plant tissues, and almonds. This approach would enhance understanding of TE behavior under varying edaphic conditions, ultimately strengthening environmental monitoring strategies and food safety regulations.

**Keywords:** Heavy metals, Quality reference values, Potentially toxic element, Environmental indicators, Natural concentration, South of Bahia

## 4.1 INTRODUCTION

The assessment of trace elements (TEs) in soils has become an essential topic in environmental sciences due to its implications for ecosystem health, agricultural productivity, human well-being, and food security (Kabata-Pendias and Mukherjee, 2007). TEs occur naturally in soils, originating from the parent material, but their concentrations can be influenced by climatic conditions, biological activity, and anthropogenic factors such as industrial emissions, agricultural inputs, and land-use changes (Brito et al., 2020; Nakazato et al., 2021; Guagliardi et al., 2022; Papazotos et al., 2024; Silva et al., 2024; Taghavi et al., 2024).

Understanding the natural background levels of these elements is crucial for distinguishing between geogenic and anthropogenic contributions, thereby aiding in environmental monitoring, pollution control, and risk assessment (Alves et al., 2024; Cardoso et al., 2024; Peirovi-Minaee et al., 2023; Weng et al., 2024). Given the growing concern over soil contamination and its potential impact on food security and public health, many countries have implemented regulatory frameworks to establish quality reference values (QRVs) for TEs in soils. In Brazil, defining QRVs on a regional scale is particularly important due to the country's vast territorial extent, diverse geology, and complex climatic variations (Boechat et al., 2020; Cardoso et al., 2023).

The Atlantic Forest is a vast area of tropical and subtropical forests that covers the Atlantic coast of South America, occupying a territory of 1,422,660 km<sup>2</sup>, known for its high levels of biodiversity and endemism, stretching from Argentina to Paraguay (Resende et al., 2024). Within its territory, 92 % is in Brazilian soil, distributed across seventeen states. Due to overexploitation, it now occupies approximately 9 % of its original area (SOSMA, 2021).

Being a coastal tropical forest, the Brazilian Atlantic Forest is located in peri-urban areas, mostly in small and isolated forest fragments dispersed mainly in agricultural and urban areas and has been undergoing profound anthropogenic changes for centuries (Carvalho et al., 2022). The region stands out nationally for its extensive cacao planting area, where about 80 % of cacao bean producers are small or medium-sized, and, for the most part, cacao is no longer a monoculture. In this sense, characterizing the natural concentration of trace elements (TEs) in soils is essential for establishing quality standards, serving as the first step in defining public policies and regulations for environmental monitoring (Chai et al., 2015; Gonçalves et al., 2022), food safety, and health effects due

to exposure and/or consumption (Boechat et al., 2016; Peirovi-Minaee et al., 2023; Silva et al., 2024b; Taghavi et al., 2024), being a practice adopted in various countries in recent decades (Alfaro et al., 2015; Laribi et al., 2023; Reimann and Caritat, 2017).

Brazilian legislation, in a regulatory approach, has established that quality reference values (QRVs) should be determined in each state and the Federal District to designate geochemical background values, based on the 75th or 90th percentiles of TEs concentrations in soils (Conselho Nacional do Meio Ambiente - CONAMA, 2009). These values should be based on the natural concentration of the element, considering soil properties, unaffected or minimally affected by anthropogenic activities (Bocardi et al., 2020).

Considering the pedodiversity of Brazilian territory, it is crucial to evaluate the natural concentrations of TEs on a regional scale to accurately delineate the characteristics and origins of elements in soils. With the need for more precise environmental monitoring, the Atlantic Forest in the southern region of the state of Bahia has the highest biodiversity indices in the country, with up to 456 tree species recorded per hectare (Araújo et al., 1998). Its preservation should not only be measured in terms of tree canopy quality but also in the quality and maintenance of natural soil characteristics. Hence, determining natural concentrations of TEs and establishing guideline values for the studied region are crucial.

Similar to China and Brazil, with vast territories, diverse geology, different climatic types, among other regional or local characteristics, environmental monitoring has demanded the use of increasingly smaller scales and higher sample densities (Brito et al., 2020; Cardoso et al., 2023; Weng et al., 2024) to obtain greater representativeness of local characteristics and avoid doubtful interpretations or the overestimation/underestimation of potential risks associated with TEs (Cardoso et al., 2024; Reimann & Garrett, 2005).

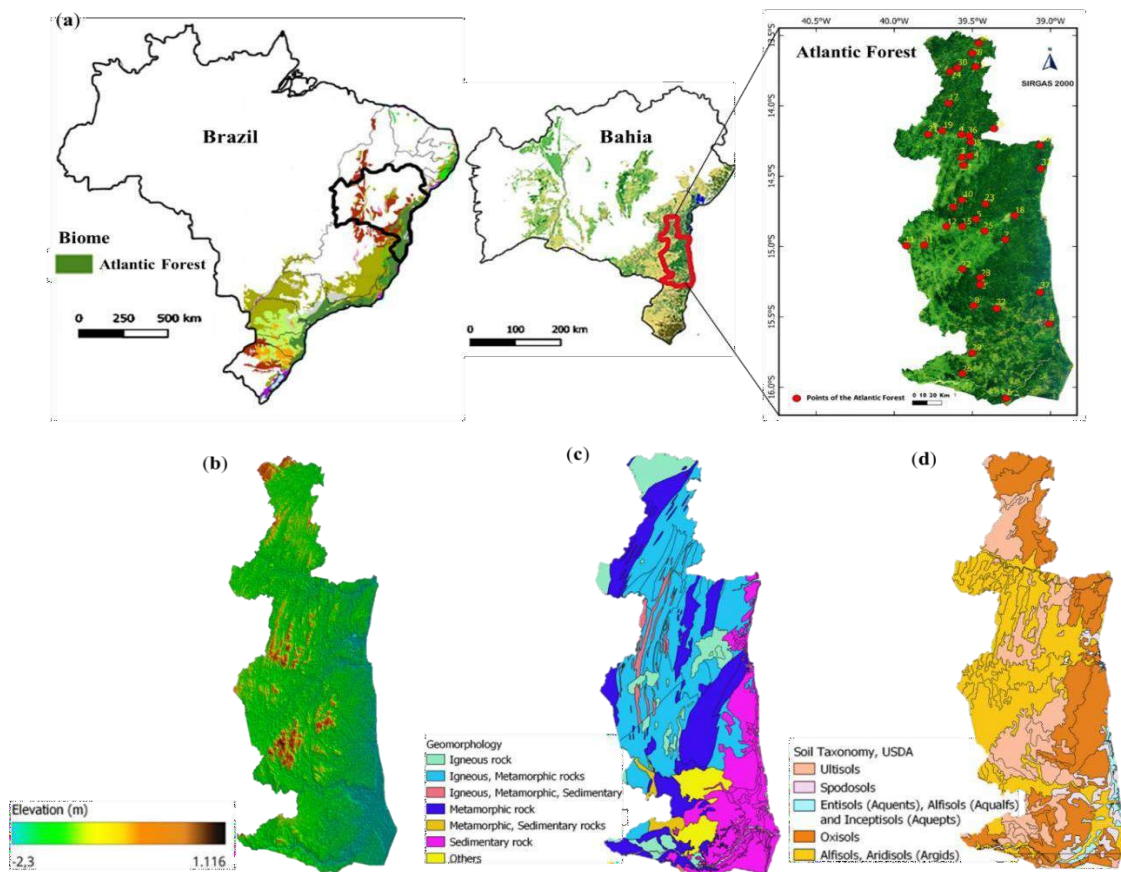
Based on the aspects discussed above and given the socio-economic and ecological importance of the Atlantic biome and the lack of evaluative data on natural levels of TEs in soils in the southern region of Bahia state, we hypothesize that in Atlantic Forest fragments without or with minimal anthropogenic intervention, concentrations of TEs in soils differ from those in other regions and can be used to establish quality reference values (QRVs). Therefore, the objectives of this study are to determine the natural concentrations of Ba, Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Sb, Ti, V, and Zn in soils under native vegetation, establish QRVs, and assess the relationship between TEs and soil

attributes. By providing baseline data on trace element concentrations, this study supports the development of region-specific environmental policies, enhances environmental quality assessments, and contributes to sustainable agricultural practices. Ultimately, it aids in the conservation of the Atlantic Forest, the well-being of local communities, food safety, and human health protection, ensuring soil quality and the safety of produced food.

## 4.2. MATERIALS AND METHOD

### 4.2.1 Study area

The study area consists of fragments of the Atlantic Forest located in the southern region of the state of Bahia, in Northeast Brazil, known as the Cacao Region (21,061 km<sup>2</sup>) (Fig. 1a). This region is composed of 41 municipalities, ranging in longitude from 39°0' to 40°5' W and in latitude from 13°5' to 16°0' S, with a population of 949,916 inhabitants. Notable municipalities include Itabuna (186,708 inhabitants) and Ilhéus (178,703 inhabitants), which serve as regional centers and hubs for economic activities and services (Instituto Brasileiro de Geografia e Estatística – IBGE, 2024).



**Figure 1** - Location map of the study area. (a) sampling points in the Atlantic Forest, located in the southern region of Bahia, Brazil, (b) elevation, (c) geology, and (d) soil types.

The lithology of the region (Fig. 1c) dates back to the Proterozoic and Archean eons, featuring very ancient rock formations belonging to the Sao Francisco Craton under the Itabuna-Salvador-Curaçá orogeny. It comprises tonalites, charnockites, monzodiorites, and belts of supracrustal rocks. The region's rocks exhibit high diversity, ranging from gabbroic, quartz monzonitic, and granodioritic to granitic (Oliveira et al., 2010), found in igneous, metamorphic, and sedimentary rocks. The soil in this region (Fig. 1d) varies widely, predominantly comprising Oxisols, Alfisols, and Ultisols (Soil Survey Staff, 2014). Despite their depth, most soils are of low natural fertility, with the more fertile soils being utilized for cacao cultivation (Andrade and Rocha, 2003).

Belonging to the Eastern Atlantic watershed, the Atlantic Forest in the southern region of Bahia has high water availability and is traversed by the Contas, Almada, and Jequitinhonha rivers. According to the Koppen-Geiger classification (Koppen, 1948), the predominant climate is tropical: equatorial (Af) along the coast, savanna (Aw) to the west of the coast, and monsoon (Am) in a continuous strip from north to south (Alvares et al., 2013). Average annual temperatures range between 21 °C and 24 °C, with monthly averages above 18 °C throughout the year.

From the coast inland and from north to south, rainfall follows a decreasing gradient, with annual totals exceeding 1,000 mm and reaching up to 1,800 mm in some locations (Mori et al., 1983). Rainfall typically occurs year-round, with no defined dry season, and the average monthly precipitation is greater than or equal to 60 mm (Milde and Nitzsche, 1985). The altitude of the region varies from east to west between -2.3 and 1,160 meters (Fig. 1b), characterized by predominantly gentle relief with hills and plains shaped by erosive processes over time.

The original vegetation of this region is predominantly evergreen tropical forest (Atlantic Forest), which once covered 15% of Brazilian territory. Today, it is the most devastated biome in the country, with only 29% of its original coverage remaining intact (SOSMA, 2021). This fragmentation has a direct impact on biodiversity (Haddad et al., 2015), vegetation structure (Câmara et al., 2017), microclimate (Schmidt et al., 2017), seed dispersal (Emer et al., 2018), and population dynamics (Arroyo-Rodríguez et al., 2015). The remaining Atlantic Forest exists in small fragments (Ranta et al., 1998) that are isolated and unevenly distributed, mostly smaller than 50 hectares and located on

private land (80%) (Ribeiro et al., 2009).

#### **4.2.2 Soil sampling and laboratory analysis**

A total of 38 composite soil samples were collected from the 0.0–0.20 m layer, formed from 5 simple samples collected in a compass pattern (north, south, east, and west, all spaced 5 m from the center of the compass) using a Dutch auger and stainless- steel shovels in areas with no or minimal anthropogenic intervention. After each collection, the geographical coordinates of each sampling point were obtained using a Garmin® GPS device. From the survey, an irregular grid was obtained, as the sampling points were chosen based on pedogeochemical variability, geopolitical distribution, and the presence of native vegetation fragments (Fig. 1a).

Chemical and granulometric analyses were performed on air-dried fine earth (ADFE), sieved through a 2.0 mm mesh sieve according to the methodology recommended by Raij et al. (2001). The pH was determined in H<sub>2</sub>O (1:2.5 v/v soil/solution), and quantifiable levels of P and K were chemically characterized using a Mehlich-1 extractor (hydrochloric acid and sulfuric acid), while Ca, Mg, and Al were characterized by titration after extraction with KCl (1 mol L<sup>-1</sup>). The potential acidity (H + Al) was extracted using calcium acetate at pH 7.0. Additionally, the cation exchange capacity (CEC) and base saturation (BS) were determined to establish the base saturation percentage (V%) and the aluminum saturation index (m%) of the samples. Total organic carbon (TOC) was determined using the Walkley-Black method by wet oxidation with 0.167 mol L<sup>-1</sup> K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and H<sub>2</sub>SO<sub>4</sub> in an acidic medium. Granulometric analyses were performed using the pipette method, which involves suspending soil in water, agitation, controlled sedimentation, and extraction of fractions using calibrated pipettes (Teixeira et al., 2017).

#### **4.2.3. Trace elements and analytical quality control**

The concentrations of the trace elements (TEs) Ba, Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Sb, Ti, V, and Zn were determined in soil samples (0.005 g) dried in an oven, ground in an agate mortar, sieved through a 0.15 mm stainless steel mesh, weighed on an analytical balance, and placed in Teflon tubes. Subsequently, 12 mL of HNO<sub>3</sub>/HCl (3:1 v/v) acid solution, both of high analytical purity (Merck PA), was added, according to the EPA

method 3051A (USEPA, 2007), in compliance with the National Environment Council (CONAMA, 2009). The samples were digested in a closed system in a microwave oven (Ethos Easy, Miles Tonesrl). The samples were pre-digested for 20 min, reaching a temperature of 175 °C for 4 min and 30 s. After cooling, the extracts were filtered using slow filtration paper (Macherey Nagel®), transferred to certified 25 mL volumetric flasks (NBR ISO/IEC), and topped up with ultrapure water (Mili-Q, 18.2 MΩ.cm). The concentrations of TEs in the extracts were determined by inductively coupled plasma optical emission spectrometry (ICP-OES Perkin Elmer 7000 DV) with an automatic sampler injection system. Calibration curves were prepared using a multielement standard solution of 1000 mg L<sup>-1</sup> (Titrisol®, Merck, Sigma-Aldrich). The quality of the analyses was verified using blank tests and certified soil sample SRM 2709 - San Joaquin Soil (NIST, 2002).

#### **4.2.4 Data set processing and statistical analysis**

Data variability was verified using univariate descriptive statistics (mean, median, minimum, maximum, skewness, kurtosis, and standard deviation). The data were tested for error normality using the Kolmogorov-Smirnov test and were considered non-parametric. Soil quality reference values (QRVs) were determined based on the 75th and 90th percentiles of the sample set, and outliers were eliminated through box-plot analysis according to Conama (2009) recommendations. A correlation matrix was developed to understand the relationships between TEs and soil characteristics. The Spearman correlation coefficient was used for data without normal distribution to evaluate the strength and direction of the association between two variables. Principal component analysis (PCA) was performed to select the soil characteristics affecting natural TEs concentrations in terms of variability and main sources. Statistical analyses were conducted using SPSS (IBM, 2020), R studio (R Core Team, 2021), and XLSTAT (Addinsoft, 2024).

### **4.3 RESULTS AND DISCUSSION**

#### **4.3.1 Analysis quality assurance**

The EPA 3051A method does not aim for complete sample decomposition, but

rather for the evaluation of environmentally available concentrations of trace elements (TEs) (Silva et al., 2014). Therefore, the National Institute of Standards and Technology (NIST) suggests that EPA 3051A method results should be compared with leachate-based element recoveries rather than total values (Biondi et al., 2011; Preston et al., 2014).

The recovery rates of the certified soil based on leachate (Table 1) were satisfactory for the metals analyzed in this study, ranging from 75 to 112%. They can be classified in ascending order (mg kg<sup>-1</sup>): Mo (0.70) < Sb (1.48) < Cd (2.68) < Pb (6.13) < Ni (6.68) < Co (6.80) < Cu (12.10) < Zn (17.70) < Fe (24.0) < Cr (36.32) < V (37.43) < Ba (66.84) < Ti (242.76). Exceptions were Cd (2.68 mg kg<sup>-1</sup>) and Ti (242.76 mg kg<sup>-1</sup>), which showed concentrations above the certified values, possibly due to natural processes (Kubier et al., 2019).

**Table 1-** Recovery rates of trace elements (TEs) in the certified soil sample SRM 2709 - San Joaquin.

TEs	VD	CV (NIST)	RD	RBL (NIST)	RBOL
	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	%	%	%
Ba	349.4	979	36	39	91
Cd	0.35	0.37	95	110	86
Co	7.91	12.8	62	81	76
Cr	56.2	130	43	41	105
Cu	26.3	33.9	78	81	96
Fe	26.2	33.6	78	70	112
Mo	1.41	nd	nd	nd	Nd
Ni	48.8	85	57	77	75
Pb	7.98	17.3	46	53	87
Sb	1.47	1.55	95	88	108
V	49.6	110	45	44	103
Zn	63	103	61	77	79

CV (NIST) = certified value by the National Institute of Standards and Technology. RD, % recovery (determined) = (determined value/certified value) × 100. RBL (NIST), % recovery by leachate = (median of leachate/certified value) × 100, RBOL. % recovery (determined) based on leachate = (determined recovery/recovery by leachate) × 100. Nd = not determined.

#### 4.3.2 Soil particles sizes and chemical characteristics

The high variability in the chemical attributes and granulometry of the soil samples corroborates the significant diversity in soil classes, geological characteristics, and climate of the region (Figs. 1c and 1d). Measures of central tendency, such as mean and median, are close for most parameters, indicating a symmetric distribution for pH, Ca, K, H + Al (potential acidity), SB (sum of bases), CEC (cation exchange capacity), base saturation (V%), sand, silt, and clay. This suggests a uniform distribution of the data around the central measures, and the skewness coefficients close to zero for these parameters reinforce the symmetry of this distribution. For the other parameters, the distribution is positively skewed (right skewed), implying a higher concentration of values below the mean (Table 2).

**Table 2** - Chemical attributes and granulometry of soil samples under Atlantic Forest, located in the southern region of Bahia (n = 38).

Parameters	Minimum	Median	Mean	Maximum	Variance	Skewness	Kurtosis	SD
pH H <sub>2</sub> O	4.52	5.8	5.66	7.94	0.42	0	-0.83	0.65
Ca (cmol <sub>c</sub> dm <sup>-3</sup> )	0.44	4.42	4.66	14.01	10.1	0.54	-0.41	3.17
Mg (cmol <sub>c</sub> dm <sup>-3</sup> )	0.45	3.24	4.03	12.93	12.09	1.39	1.25	3.48
K (cmol <sub>c</sub> dm <sup>-3</sup> )	0.04	0.23	0.27	0.66	0.03	0.97	0.08	0.16
P (mg dm <sup>-3</sup> )	5.45	13.63	23.77	144.77	771.2	2.17	4.54	27.7
Al (cmol <sub>c</sub> dm <sup>-3</sup> )	0.0	0.0	0.44	2.25	0.42	1.53	1.55	0.65
H + Al (cmol <sub>c</sub> dm <sup>-3</sup> )	0.73	3.26	3.82	9.1	5.68	0.56	0.74	2.38
SB (cmol <sub>c</sub> dm <sup>-3</sup> )	1.01	8.76	8.94	25.7	37.16	0.56	-0.45	6.09
CEC (cmol <sub>c</sub> dm <sup>-3</sup> )	2.94	12.14	12.71	31.66	38.14	0.65	0.15	6.17
V% (%)	15.26	74.14	65.35	94.86	592.2	-0.66	-0.87	24.3
m% (%)	0.0	0.0	11.2	69.45	322.1	1.75	2.39	17.9
TOC (g kg <sup>-1</sup> )	5.27	22.94	27.12	82.81	273.07	1.11	1.3	16.5
sand (g kg <sup>-1</sup> )	196.26	499.23	510.4	822.88	26.845	0.09	-0.57	163
silt (g kg <sup>-1</sup> )	11.42	220.5	231.57	595.95	15.073	1.05	1.66	122
clay (g kg <sup>-1</sup> )	49.74	227.77	258.03	563.42	18.269	0.67	-0.33	135

n = number of samples. H + Al = potential acidity. SB = sum of bases. CEC = cation exchange capacity. V% = base saturation. m% = aluminum saturation index. TOC = total organic carbon. SD = Standard Deviation.

In the calculation of kurtosis, most parameters exhibited a platykurtic curve, with values of  $C < 3$ , pH, Ca, Mg, K, Al, H + Al, Al, V%, m%, TOC, sand, silt, and clay, indicating a broader dispersion of the data around the mean. However, phosphorus (P) shows a leptokurtic curve, with values of  $C > 3$ , indicating a more concentrated

distribution around the mean, which may indicate less variability of this element in the studied soils. Regarding soil granulometry, the standard deviation evidences a greater dispersion of the data analyzed, suggesting significant variability in this soil characteristic. This greater dispersion can be attributed to factors such as variations in soil texture at different sampling locations.

The average soil pH in water was 5.66, ranging from 4.52 to 7.94, indicating moderately acidic to alkaline conditions. The concentrations of available aluminum (0.00 to 2.25  $\text{cmol}_c \text{dm}^{-3}$ ), potential acidity (0.73 to 9.10  $\text{cmol}_c \text{dm}^{-3}$ ), and Al saturation (0.00 to 69.45%) are considered low to very high. Consequently, acidic soil conditions may increase environmental risks associated with aluminum (Al) availability, as it is the predominant exchangeable cation in acidic Brazilian soils (Abreu Jr. et al., 2003). The average concentrations of calcium (Ca), magnesium (Mg), and potassium (K) were 4.66, 4.03, and 0.27  $\text{cmol}_c \text{dm}^{-3}$ , respectively, with available concentrations varying: Ca (0.44 to 14.01  $\text{cmol}_c \text{dm}^{-3}$ ), Mg (0.45 to 12.93  $\text{cmol}_c \text{dm}^{-3}$ ), and K (0.04 to 0.66  $\text{cmol}_c \text{dm}^{-3}$ ), showing high variability between the minimum and maximum values of these elements in the soil. The presence of available P in the soil was low (23.77  $\text{cmol}_c \text{dm}^{-3}$  on average).

The average total organic carbon content of the soil was 27.12  $\text{g kg}^{-1}$ , ranging from 5.27 to 82.81  $\text{g kg}^{-1}$ , with concentrations varying from medium to high, justified by the senescence of branches and leaves that compose the typical litter of forested areas in the rhizosphere layer of the soil. The variability in TOC levels in remnants of the Atlantic Forest in Brazil is high and has been observed in other studies (Monroe et al., 2022; Oliveira et al., 2019; Silva et al., 2012). This is justified by the fact that, even in conservation areas, the history of occurrence of wildfires is often unknown, leading to carbon loss in the surface layers.

The clay content of the analyzed soils ranged from 49.74 to 563.42  $\text{g kg}^{-1}$ , and the average sand content was 510.40  $\text{g kg}^{-1}$ , highlighting the high granulometric variation of the region, with medium-textured soils (71%), clayey soils (23.7%), and extremely sandy soils (5.3%). The disparity between the minimum and maximum nutrient and texture contents of the evaluated soils is directly reflected in the calculated variables SB, CEC, V%, and m% (Table 2).

### **4.3.3 TEs natural concentrations**

The concentrations of TEs in the studied region were mostly lower than those

described for soils in the international literature, Štrbac et al. (2022) and Alloway (1990), except for Cd (2.68 mg kg<sup>-1</sup>), compared to global soils (Table 3). When compared to soils from the northern and northeastern regions of Brazil: Ba and Cd are higher than reported in all states, except for Amazonas (Lima et al., 2020). In regional assessments, the river basins of Bahia (Cardoso et al., 2023) and Piauí (Brito et al., 2020), in addition to Cd, other elements such as Ba, Co, Cu, Fe, Mo, Pb, Sb, V, and Zn also showed high levels, except for Cr and Ni (Table 3).

In the soils of the Atlantic Forest in the southeastern region (Nakazato et al., 2021), Ba and Cd were the only elements that maintained a high concentration in natural levels. The high concentrations of Cd may be associated with the weathering of granitic, gabbroic, sedimentary and intrusive rocks (rich in Cu sulfide). In general, Cd concentrations in sedimentary rocks (0.01 to 2.6 mg kg<sup>-1</sup>) are higher than those in igneous rocks (0.07 to 0.25 mg kg<sup>-1</sup>) or metamorphic rocks (0.11 to 1.0 mg kg<sup>-1</sup>) (Kubier et al., 2019; Mar and Okazaki, 2012), which increase the concentration of this element in the soil (Fig. 1c). Also, in a scientific study evaluating soils of the Brazilian Cerrado formed under different parent materials, average Ba values in ascending order of 33, 41, and 299 mg kg<sup>-1</sup> were observed in soils derived from sedimentary rock, basalt, and gneiss, respectively (Marques et al., 2004a).

**Table 3** Average concentrations of TEs (mg kg<sup>-1</sup>, Fe in g kg<sup>-1</sup>, dry soil) in Atlantic Forest soils (0.0–0.20 m), located in the southern region of Bahia, Brazil (n = 38), compared with data compiled in national and international literature.

	Ba	Cd	Co	Cr	Cu	Fe	Mo	Ni	Pb	Sb	Ti	V	Zn	
Internacional soils														
Europe	nd	2.99	11.1	38.3	22.1	nd	nd	22.5	13.6	nd	nd	nd	76.8	Štrbac et al. (2022).
Soils of the world	84-960	0.01–2.0	nd	5–1.500	2–250	nd	nd	2–750	2–750	2.0-300	nd	nd	1–900	Alloway (1990)
Brazilian soils														
North/Northeast Region														
Amazon	nd	0.1	nd	6.9	2.8	15.4	15.1	1.7	4.4	nd	nd	nd	5.7	Nascimento et al. (2018)
Amazon	nd	10.1	12.3	88.4	110	32.1	578.6	30.2	10.4	nd	nd	nd	48.5	48.5 Lima et al.(2020)
Pará	16.7	0.1	1.6	14.3	6.0	9.3	nd	1.4	10.4	nd	nd	35	35.0	CPRH (2014)
Paraíba	nd	0.06	7.9	11.2	28.8	18.7	268.3	9.1	10.0	23.4	nd	23.4	23.4	Almeida Jr. et al. (2016)
Piauí	66.2	0.03	5.1	40.1	16.6	27.6	1.5	13.8	8.6	1.4	nd	57.8	14.0	Boechat et al. (2020)
Rio Grande do Norte	58.9	0.1	nd	30.9	13.7	nd	nd	19.8	16.2	0.2	nd	nd	23.8	Preston et al. (2014)
Watershed														

Bahia	nd	0.09	4.08	40.9	10.7	24.3	nd	9.4	4.9	nd	nd	nd	28.28	Cardoso et al. (2023)
Piauí	9.1	0.05	nd	43.4	0.74	18.7	0.34	0.8	2.7	0.007	nd	47.83	0.46	Brito et al. (2020)
Atlantic Rain Forest (Southeast Region)														
Minas Gerais	nd	0.8	nd	75.8	23.8	22.6	nd	24.6	10.5	nd	nd	nd	56.6	Nakazato et al. (2021)
São Paulo	nd	2.1	nd	26.1	10.3	14.3	nd	4.5	10.6	nd	nd	nd	34.2	Nakazato et al. (2021)
Santo André	nd	0.01	nd	12.2	6.95	11.2	nd	4.7	14.4	nd	nd	nd	9.28	Nakazato et al. (2021)
Campinas	nd	0.28	nd	13.0	48.5	20.4	nd	9.2	7.6	nd	nd	nd	40.1	Nakazato et al. (2021)
Atlantic Rain Forest (Northeast Region)														
Study area	66.8	2.7	6.8	36.3	12.1	24.0	0.7	6.7	6.1	1.5	242.8	37.43	17.7	Authors

The variability observed in the soils of the Atlantic Forest, located in the southern microregion of the State of Bahia, compared to those reported in other native areas around the world, reflect the diversity of parent materials and pedogenesis processes, as well as the intrinsic characteristics of each parent rock, soil class, and analyzed element (Bini et al., 2011; Boechat et al., 2020; Cardoso et al., 2023). Such comparisons are essential due to the regional differences that must be observed to be accounted for in the monitoring plan of the Atlantic Forest biome.

#### 4.3.4 Correlation between soil characteristics and TEs concentration

The Spearman correlation coefficient ( $p > 0.05$ ) was used to evaluate the impact of soil fertility variables on the concentration of trace elements (TEs) in the soils (Fig. 2). The TEs were positively correlated with each other, suggesting a common lithogenic source, an expected result for natural forest areas with minimal anthropogenic interference. The correlations of clay with TEs were positively low or nonexistent, which can occur due to the high ion adsorption capacity of clay, including metals (Ismadji et al., 2015), influenced by pH and by the presence of exchangeable bases in the soil, which also maintained low correlations with the TEs.

TEs were positively correlated with each other, suggesting a common lithogenic source, which is an expected result for natural forest areas with minimal anthropogenic interference. In general, the correlations of pH and total organic carbon (TOC) with TEs were low positive or nonexistent, which could be explained by the high levels of Fe and Al oxides (Fig. 2; Table 3). These oxides have a high surface area and variable charge, allowing them to adsorb metal cations such as  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Co}^{2+}$ . This



negative correlations with Sb, V, Cd, and Fe, indicating that soils with higher TOC accumulation tend to be less susceptible to the concentration of these elements (Kabata-Pendias & Mukherjee, 2007). The attributes related to soil acidity (Al, H+Al, m%) exhibited negative and low correlations with most TEs (Fig. 2), which is explained by the fact that acidic soils tend to increase the solubility of certain heavy metals (Alloway, 2013). However, the soils in the analyzed region were moderately acidic to slightly alkaline (Table 2). P, in turn, did not show consistent correlation with the TEs due to competition for adsorption sites, which can both increase and decrease the availability of these metals. In this context, it is evident that the chemical process of metal adsorption in the soil is probably the most important process for the retention and release of these elements (Hooda, 2010).

The principal component analysis (PCA) for TEs concentrations and soil characteristics has been provided in Table 4. The first and second PCs cumulatively explained 64.17% of the dissimilarity between the different sampling points with PC1 = 42.42% and PC2 = 21.75%, according to the cutoff criterion (greater than 0.70 in absolute value) considered for the interpretation of each principal component.

**Table 4** Factor analysis between the original variables and the principal components of chemical elements, granulometry, and TEs of soils under the Atlantic Forest, located in the southern region of Bahia.

TEs	PC1	PC2	PC3
Ba	<b>0.694</b>	-0.417	0.37
Cd	<b>0.752</b>	0.552	-0.135
Co	<b>0.867</b>	-0.283	0.191
Cr	0.606	0.648	-0.11
Cu	<b>0.872</b>	0.174	-0.093
Fe	<b>0.758</b>	0.553	-0.138
Mo	-0.473	0.314	-0.104
Ni	<b>0.878</b>	-0.003	0.148
Pb	0.515	0.171	0.025
Sb	0.625	0.613	-0.172
Ti	<b>0.821</b>	0.236	0.161
V	<b>0.708</b>	0.531	-0.282
Zn	<b>0.913</b>	-0.114	0.184
clay	0.166	0.674	0.513
pH	0.483	-0.652	-0.409

TOC	-0.249	0.131	-0.387
Ca	0.564	-0.606	-0.125
Mg	<b>0.725</b>	-0.447	-0.129
K	0.317	-0.376	-0.294
P	0.399	-0.651	0.376
Al	-0.571	0.611	0.238
Variance explained by component	Eigenvalues	% Total variance	% Total cumulative variance
PC1	8.909	42.42	42.42
PC2	4.52	21.75	64.17
PC3	1.32	6.32	70.5

TOC = total organic carbon. Bold marking > 0.70

The variables retained in PC1 that exhibited high positive loadings included Zn (0.913), Cu (0.872), Ni (0.878), Co (0.867), Fe (0.758), Cd (0.752), Mg (0.725), V (0.708), and Ba (0.694). The strong correlation among these elements suggests they may share a similar geological origin, possibly released from specific rocks or minerals present in the studied region. The region's geology includes gabbroic rocks, which can contain sulfide minerals that release heavy metals such as Zn and Cu, and granitic rocks that may release cadmium, iron, and manganese under certain weathering conditions. In PC2, the positive correlations were weak, but clay (0.674), Cr (0.648), Al (0.611), and Sb (0.613) emerged as the main contributors, suggesting that these elements are associated with fine soil fractions, such as clay and Fe and Al oxides, which exhibit high surface area and metal retention capacity in highly weathered Brazilian soils. Antimony may be linked to the clay fraction and oxides, exhibiting low mobility, a behavior like other trace metals. PC2 was also associated with negative correlations observed between P (-0.651), pH (-0.652), and Ca (-0.606).

The negative correlation between phosphorus (P) and clay may indicate that P is strongly adsorbed onto Fe and Al oxides, reducing its availability in the soil (Silva et al., 2023). The pH correlation suggests that more acidic soils are associated with higher clay, Cr, Al, and Sb contents, reinforcing the influence of metal oxides in retaining these elements. The behavior of Ca suggests that soils with lower exchangeable base contents (such as Ca and Mg) exhibit higher clay content and metals bound to oxides. This occurs because divalent cations like Ca<sup>2+</sup> compete with metals for adsorption sites on Fe and Al oxides, potentially reducing the retention of Cr and Sb in soils with higher Ca levels

(Marques et al., 2004b).

Considering the variability of soil chemical properties in the region (Fig. 1; Tables 2 and 3), we suggest that more clayey, acidic soils with low Ca content tend to accumulate these metals, whereas soils with higher pH and Ca levels may reduce Cr and Sb adsorption, thus enhancing their mobility.

#### 4.3.5 Quality reference values for TEs

The reference quality values (QRVs) for TEs in the soils of the Atlantic Forest, located in the southern region of the State of Bahia, are presented according to the 75th percentile (P75) and classified in ascending order, mg kg<sup>-1</sup>: Mo (0.13) < Sb (1.99) < Cd (3.47) < Pb (7.81) < Ni (11.45) < Co (12.50) < Cu (16.99) < Zn (24.02) < V (50.12) < Cr (54.24) < Ba (116.11) < Ti (357.20) < Fe (32,740) (Table 5). Generally, the geological processes of rock degradation forming the soils on very ancient surfaces, such as those in the studied region (Proterozoic and Archean domains), commonly contain high concentrations of TEs due to the prolonged weathering process (Alloway, 2013; Cardoso et al., 2023; Gonçalves et al., 2022; Oliveira, 2010).

**Table 5** Descriptive statistics and soil quality reference values (QRVs) for TEs in soils under the Atlantic Forest, located in the southern region of Bahia, Brazil.

Descriptive statistics	Ba	Cd	Co	Cr	Cu	Fe	Mo	Ni	Pb	Sb	Ti	V	Zn
	mg kg <sup>-1</sup>												
Mean	66.84	2.68	6.8	36.32	12.1	24,089	0.7	6.68	6.13	1.48	242.76	37.43	17.7
Median	51.27	2.74	3.39	35.29	9.24	24,099	0.05	4.47	5.79	1.41	218.33	39.49	16.9
Minimum	2.97	0.4	0.35	6.09	3.25	3,859	bdl	0.75	2.73	0.32	bdl	7	5.73
Maximum	234.02	6.1	28.2	80.44	32.3	54,749	0.28	16.85	11.98	3.32	618.98	71.25	37.34
Standard	63.12	1.54	7.28	19.98	7.63	13,717	0.07	5.05	2.49	0.71	172.62	17.33	9.65
Skewness	0.94	0.45	1.17	0.16	0.78	0.44	0.8	0.55	0.6	0.39	0.51	0	0.48
Kurtosis	-150	-0.19	0.63	-0.98	-0.12	-0.32	0.09	-1.18	-0.24	-0.35	-0.63	-0.8	-0.88
n1	35.0	36	37	37	35	36	30	36	37	37	38	33	36
n2	3.0	2	1	1	3	2	8	2	1	1	0	5	2
P (75)	116.11	3.47	12.5	54.24	16.99	32,74	0.13	11.45	7.81	1.99	357.2	50.12	24.02
P (90)	152.91	5.14	18.9	61.64	24.04	46,081	0.17	14.15	9.55	2.43	535.52	59.35	32.8

n1 = total samples for obtaining the QRV. n2 = total anomalous samples collected through box-plot observation. P (75) = QRV calculated based on the 75th percentile. P (90) = QRV calculated based on the 90th percentile. bdl = below the detection limit.

Considering the 75th percentile, in the Atlantic Forest soils of the cacao-producing region, the QRVs for all metals were higher than those calculated for the State of Pará (Gonçalves et al., 2022), except for Pb (17.8 mg kg<sup>-1</sup>) and V (57.2 mg kg<sup>-1</sup>); the State of Piauí (Boechat et al., 2020), except for Mo (2.3 mg kg<sup>-1</sup>), Ni (19.3 mg kg<sup>-1</sup>), and Pb (11.4 mg kg<sup>-1</sup>); the State of Paraíba (Almeida Jr. et al., 2016), except for Cu (28.81 mg kg<sup>-1</sup>) and Pb (10.01 mg kg<sup>-1</sup>); and the State of Rio Grande do Norte (Preston et al., 2014), except for Co (15.41 mg kg<sup>-1</sup>), Ni (19.84 mg kg<sup>-1</sup>), and Pb (16.18 mg kg<sup>-1</sup>). Additionally, we also observed higher values at the 75th percentile compared to a watershed located in the Caatinga and Atlantic Forest ecotone in the State of Bahia (Cardoso et al., 2023), except for Cr (54.51 mg kg<sup>-1</sup>) and Zn (31.66 mg kg<sup>-1</sup>).

The natural concentrations and QRVs of TEs established for the entire region appear to be governed by the diversity of pedogeochemical conditions that characterize the study area. The petrology of the analyzed region strongly influences the concentration of TEs in the soil, as it consists of an igneous suite of metagranitoids and tonalitic to granitic gneisses, with enclaves of metagabbro and metadiorite, as well as norite-pyroxenite bodies rich in Cu sulfide, mostly metamorphosed in the granulite facies (Kosin et al., 2003; Oliveira et al., 2010; Souza et al., 2020), contributing to the natural enrichment in Ba, Cd, Co, Cr, Cu, Fe, Mo, Ni, Pb, Sb, Ti, V, and Zn (Figs. 1c and 1d; Table 4).

Given the above, we suggest the need to define QRVs based on geological, geomorphological, lithological, pedological, and biome-specific contexts, due to the differences in TEs concentrations and distribution, as observed in the Atlantic Forest soils of the cacao-producing region. Moreover, these studies play a crucial role in protecting human health and food safety due to the mobility and bioavailability of metals and the risk of contaminating the food chain, negatively impacting public health. Therefore, these threshold values serve as a reference for environmental monitoring programs, aiding in the identification of contaminated soils and the implementation of mitigation measures. Thus, the proper definition of QRVs contributes to soil quality maintenance and the sustainability of agricultural production, preventing the bioaccumulation of toxic metals in ecosystems.

#### **4.4 CONCLUSION**

The study demonstrates that the concentrations of trace elements (TEs) in soils under the Atlantic Forest in the southern region of Bahia State differ from those found in

other regions. This unique characteristic is attributed to the area's diverse geological composition, where pedogenetic processes have led soils to inherit metals of natural geochemical origin, resulting in specific and distinct TEs concentrations, following the order: Fe > Ti > Ba > V > Cr > Zn > Cu > Co > Ni > Pb > Cd > Sb > Mo. The primary factor governing TEs concentrations in soils is the parent material.

The quality reference values (QRVs) for TEs were determined according to the 75th percentiles (P75), classified in ascending order (mg kg<sup>-1</sup>): Mo (0.13) < Sb (1.99) < Cd (3.47) < Pb (7.81) < Ni (11.45) < Co (12.50) < Cu (16.99) < Zn (24.02) < V (50.12) < Cr (54.24) < Ba (116.11) < Ti (357.20) < Fe (32,740), and the 90th percentiles (P90) (mg kg<sup>-1</sup>): Mo (0.17) < Sb (2.43) < Cd (5.14) < Pb (9.55) < Ni (14.15) < Co (18.94) < Cu (24.04) < Zn (32.8) < V (59.35) < Cr (61.64) < Ba (152.91) < Ti (535.52) < Fe (46,081).

The irregular topography, dense vegetation, geological composition, and climatic weathering influence the pedogenesis of the Atlantic Forest, thereby affecting TEs mobility. Establishing regionally specific QRVs for TEs proved to be an effective strategy for environmental monitoring, as the observed differences compared to other Brazilian regions and international standards are significant. Thus, environmental agencies and local institutions will have reference values to develop more localized surveillance and monitoring strategies, aligning with the region's intrinsic characteristics.

Furthermore, for future research, it is crucial to expand spatial analyses of QRVs across different Brazilian biomes, particularly in regions with intensive agricultural activity and high environmental sensitivity. Investigating the long-term impacts of land use changes on TEs dynamics and their potential bioavailability in the food chain could provide valuable insights for soil conservation policies. Lastly, integrating ecological and human health risk indices in cacao-cultivated areas, considering soil, litter, plant tissues, and fruits (almonds, the commercial product), could enhance knowledge about TEs behavior under different edaphic conditions, ultimately improving environmental monitoring strategies and food safety assessments.

#### 4.5 REFERENCES

Abreu Jr., C. H., Muraoka, T., & Lavorante, A. F. (2003). Relationship between acidity and chemical properties of brazilian soils. *Scientia Agricola*, 60, 337-343. <https://doi.org/10.1590/S0103-90162003000200019>. Addinsoft. (2024). XLSTAT statistical and data analysis solution (Version free trial). Addinsoft. <https://www.xlstat.com>.

Agência Estadual de Meio Ambiente – CPRH. (2014). *Instrução Normativa N° 007/2014: Estabelece os valores de referência da qualidade do solo (VRQ) do Estado de Pernambuco quanto à presença de substâncias químicas para o gerenciamento ambiental de áreas contaminadas por essas substâncias*. Retrieved May 27, 2024, from <https://www.legisweb.com.br/legislacao/?id=279789>.

Alfaro, M. R., Montero, A., Ugarte, O. M., do Nascimento, C. W. A., Accioly, A. M. A., Biondi, C. M., & Silva, Y. J. A. B. (2015). Background concentrations and reference values for heavy metals in soils of Cuba. *Environmental Monitoring and Assessment*, 187, 4198. <https://doi.org/10.1007/s10661-014-4198-3>

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M., & Sparovek, G. (2013). Kooppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22, 711–728. <https://doi.org/10.1127/0941-2948/2013/0507>.

Alves, P. N., Cardoso, K. M., Nascimento, C. W. A., Barros, J. S., Sena, A. F. S., Morais, P. G. C., Saraiva, P. C., Escobar, M. E. O., Cunha, K. P. V., & Boechat, C. L. (2024). Heavy metals in soils derived from sedimentary rocks of the Gurgueia River watershed, Northeast, Brazil: background values, distribution and ecological risk assessment. *Environmental Geochemistry and Health*, 46, 438. <https://doi.org/10.1007/s10653-024-02216-8>.

Almeida Júnior, A. B., Nascimento, C. W. A., Biondi, C. M., Souza, A. P., & Barros, F.M. R. (2016). Background and reference values of metals in soil from Paraíba State. Brazil. *Revista Brasileira de Ciência do Solo*, 40, 1–13. <https://doi.org/10.1590/18069657rbcs20150122>

Alloway, B. J. (1990). *Heavy Metals in Soils*. Blackie Academic and Professional.

Alloway, B. J. (2013). *Heavy metals in soils*. third ed. Springer. Andrade, M. P., & Rocha, L. B. (2003). *Ilhéus: passado e presente*. second ed. Editus.

Araújo, M., Alger, K., Mesquita, C. A. B., & Rocha, R. (1998). *A Mata Atlântica do Sul da Bahia: Situação atual, ações e perspectivas*. Caderno 08. CNRBA.

Arroyo-Rodríguez, V., Melo, F. P. L., Martínez-Ramos, M., Bongers, F., Chazdon, R. L., Meave, J. A., & Tabarelli, M. (2015). Multiple successional paths in human-modified landscapes: new insights into forest succession, forest fragmentation, and landscape ecology research. *Biological reviews of the Cambridge Philosophical Society*, 92, 326–340. <https://doi.org/10.1111/brv.12231>

Bini, C., Sartori, G., Wahsha, M., & Fontana, S. (2011). Background levels of trace elements and soil geochemistry at regional level in NE Italy. *Journal of Geochemical Exploration*, 109, 125–133. <https://doi.org/10.1016/j.gexplo.2010.07.008>

Biondi, C. M., Nascimento, C. W. A., Neta, A. B. F., & Ribeiro, M. R. (2011). Teores de Fe, Mn, Zn, Cu, Ni e Co em solos de referência de Pernambuco. *Revista Brasileira de Ciência do Solo*, 35, 1057–1066. <https://doi.org/10.1590/S0100-06832011000300039>

- Bocardi, J. M. B., Pletsch, A. L., Melo, V. F., & Quinaia, S. P. (2020). Quality reference values for heavy metals in soils developed from basic rocks under tropical conditions. *Journal of Geochemical Exploration*, 217. <https://doi.org/10.1016/j.gexplo.2020.106591>
- Boechat, C. L., Pistoia, V. C., Gianelo, C., & Camargo, F. A. O. (2016). Accumulation and translocation of heavy metals by spontaneous plants growing in a site contaminated with multimetallics in the southeastern state of Rio Grande do Sul, Brazil. *Environmental Science and Pollution Research*, 23, 2371-2380. <https://doi.org/10.1007/s11356-015-5342-5>
- Boechat, C. L., Duarte, L. S. L., Sena, A. F. S., Nascimento, C. W. A., Silva, Y. J. A. B., Brito, A. C. C., & Saraiva, P. C. (2020). Background concentrations and quality reference values for potentially toxic elements in soils of Piauí state, Brazil. *Environmental Monitoring and Assessment*, 192, 723. <https://doi.org/10.1007/s10661-020-08656-w>
- Brito, A. C. C., Boechat, C. L., Sena, A. F. S., Duarte, L. S. L., Nascimento, C. W. A., Silva, Y. J. A. B., Silva, Y. J. A. B., & Saraiva, P. C. (2020). Assessing the distribution and concentration of heavy metals in soils of an agricultural frontier in the Brazilian Cerrado. *Water, Air, & Soil Pollution*, 231, 388. <https://doi.org/10.1007/s11270-020-04760-2>
- Câmara, T., Almeida, W. R., Tabarelli, M., Andersen, A. N., & Leal, I. R. (2017). Habitat fragmentation, NEF-bearing trees and ant communities: Ecological cascades in the Atlantic Forest of Northeast Brazil. *Southern Ecology*, 42, 31-39. <https://doi.org/10.1111/aec.12393>
- Cardoso, K. M., Boechat, C. L., Nascimento, C. W. A., Escobar, M. E. O., Silva, D. G., Lins, S. A. S., & Morais, P. G. C. (2023). Watershed-scale assessment of the environmental values of potentially toxic soil elements of the Caatinga and Atlantic Forest ecotone in Brazil. *Chemosphere*, 338, 139394. <https://doi.org/10.1016/j.chemosphere.2023.139394>
- Cardoso, K. M., Nascimento, C. W. A., Lins, S. A. S., Nascimento, C. C., Oliveira, R. L., Silva, D. G., Morais, P. G. C., & Boechat, C. L. (2024). Assessing ecological risks and spatial distribution of potentially toxic elements in soils from anthropized environments in a watershed at the caatinga-Atlantic Forest ecotone in Brazil. *Environmental Research*, 249, 118423. <http://doi.org/10.1016/j.envres.2024.118423>.
- Carvalho, G. O. T. D., Silva, N. C. D., & Salvio, G. M. M. (2022). Vulnerabilidade ambiental em Áreas de Proteção Ambiental (APA) do Bioma Mata Atlântica na região sudeste brasileira. *Ciência Florestal*, 32, 1575-1593. <https://doi.org/10.5902/1980509867261>
- Chai, Y., Guo, J., Chai, S., Cai, J., Xue, L., & Zhang, Q. (2015). Identification of eight heavy metals in pasture soils by multivariate analysis of the Baicheng-Songyuan area, Jilin Province, Northeast China. *Chemosphere*, 134, 67-75. <https://doi.org/10.1016/j.chemosphere.2015.04.008>
- Conselho Nacional do Meio Ambiente - CONAMA. (2009). Resolução N. 420 de 28 de fevereiro de 2009. Retrieved January 25, 2024, from <http://www.mma.gov.br/port/conama/res/res09/res42009.pdf>.

Emer, C., Galetti, M., Pizo, M. A., Guimarães Jr, P. R., Moraes, S., Piratelli, A., & Jordano, P. (2018). Seed dispersal interactions in fragmented landscapes – a meta- lattice approach. *Ecology Letters*, 21, 484-493. <https://doi.org/10.1111/ele.12909>

Gomes, P. C., Fontes, M. P. F., Silva, A. G., Mendonça, E. S., & Netto, A. R. (2001). Selectivity sequence and competitive adsorption of heavy metals by Brazilian soils. *Soil Science Society of America Journal*, 65, 1115–1121. <https://doi.org/10.2136/sssaj2001.6541115x>

Gonçalves, D. A. M., Pereira, W. V. S., Johannesson, K. H., Pérez, D. V., Guilherme, L.R. G., & Fernandes, A. R. (2022). Geochemical background for potentially toxic elements in forested soils of the State of Pará, Brazilian Amazon. *Minerals*, 12, 674. <https://doi.org/10.3390/min12060674>

Guagliardi, I., Astel, A. M., & Cicchella, D. (2022). Exploring soil pollution patterns using self-organizing maps. *Toxics*, 10, 416. <https://doi.org/10.3390/toxics10080416>

Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., & Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, 1, e1500052. <https://doi.org/10.1126/sciadv.1500052>

Hooda, P. S. (2010). *Trace elements in soils*. John Wiley and Sons.

IBM Corp. (2020). IBM SPSS Statistics for Windows, Version Statistics 22.0. IBM Corp.

Instituto Brasileiro de Geografia e Estatística – IBGE. (2024). Levantamento Sistemático da Produção Agrícola - Área plantada, área colhida e produção, por ano da safra e produto das lavouras. Retrieved February 05, 2024, from <https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9201-levantamento-sistematico-da-producao-agricola.html>.

Ismadji, S., Soetaredjo, F. E., & Ayucitra, A. (2015). *Clay Materials for Environmental Remediation*. Springer. Kabata-Pendias, A.; Mukherjee, A.B. (2007). Trace elements from soil to human. first ed. Springer, Berlin. Köppen W. (1948). *Climatologia: com um estudo de los climas de la tierra*. Laboratory of Climatology.

Kosin, M., Melo, R. C., Souza, J. D., Oliveira, E. P., Carvalho, M. J., & Leite, C. M. (2003). Geologia do segmento norte do Orógeno Itabuna-Salvador-Curaçá e guia de excursão. *Brazilian Journal of Geology*, 33, 15-26. <https://doi.org/10.25249/0375-7536.200333S11526>

Kubier, A., Wilkin, R. T., & Pichler, T. (2019). Cadmium in soils and groundwater: A review. *Applied Geochemistry*, 108, 104388. <https://doi.org/10.1016/j.apgeochem.2019.104388>.

Laribi, A., Shand, C., Wendler, R., Mouhouche, B., Hillier, S., & Colinet, G. (2023). Ambient background and quality reference values for trace metals in soils from Algeria. *Soil and Water Research*, 18, 33–42. <https://doi.org/10.17221/143/2021-SWR>

- Lima, M. W., Hamid, S. S., Souza, E. S., Teixeira, R. A., Palheta, D. C., Faial, K. C. F., & Fernandes, A. R. F. (2020). Geochemical background concentrations of potentially toxic elements in soils of the Carajás Mineral Province, southeast of the Amazonian Craton. *Environmental Monitoring and Assessment*, 192, 649–664. <https://doi.org/10.3390/min12060674>
- Lin, J., Zhao, Y., Zhan, Y., & Wang, Y. (2020). Influence of coexisting calcium and magnesium ions on phosphate adsorption onto hydrous iron oxide. *Environmental Science and Pollution Research*, 27, 11303–11319. <https://doi.org/10.1007/s11356-020-07676-w>
- Mar, S. S., & Okazaki, M. (2012). Investigation of Cd contents in several phosphate rocks used for the production of fertilizer. *Microchemical Journal*, 104, 17–21. <https://doi.org/10.1016/j.microc.2012.03.020>
- Marques, J. J., Schulze, D. G., Curi, N., & Mertzman, S. A. (2004a). Trace element geochemistry in Brazilian Cerrado soils. *Geoderma*, 121, 31–43. <https://doi.org/10.1016/j.geoderma.2003.10.003>
- Marques, J. J., Schulze, D. G., Curi, N., & Mertzman, S. A. (2004b). Major element geochemistry and geomorphic relationships in Brazilian Cerrado soils. *Geoderma*, 119, 179–195. [https://doi.org/10.1016/S0016-7061\(03\)00260-X](https://doi.org/10.1016/S0016-7061(03)00260-X)
- Milde, L. C. E. & Nitzsche, M. H. (1985). Estudo de precipitação diária: I. Regimes pluviométricos da região cacauzeira da Bahia. *Revista Theobroma*, 15, 79–95
- Monroe, P. H. M., Gama-Rodrigues, E. F., Gama-Rodrigues, A. C., & Vicente, L. C. (2022). Carbon and nitrogen occluded in soil aggregates under cacao-based agroforestry systems in Southern Bahia, Brazil. *Journal of Soil Science and Plant Nutrition*, 22, 1326–1339. <https://doi.org/10.1007/s42729-021-00734-3>
- Mori, S. A., Boom, B. M., Carvalho, A. M., & Santos, T. S. (1983). Southern Bahian moist forests. *The Botanical Review*, 49, 155–232. <https://doi.org/10.1007/BF028610>
- Nakazato, R. K., Lourenço, I. S., Esposito, M. P., Lima, M. E. L., & Ferreira, M. L. (2021). Trace metals at the tree-litter-soil- interface in Brazilian Atlantic Forest plots surrounded by sources of air pollution. *Environmental Pollution*, 268, 115797. <https://doi.org/10.1016/j.envpol.2020.115797>
- Nascimento, C. W. A., Lima, L. H. V., Silva, F. L., Biondi, C. M., & Campos, M. C. C. (2018). Natural concentrations and reference values of heavy metals in sedimentary soils in the Brazilian Amazon. *Environmental Monitoring and Assessment*, 190, 606–614. <https://doi.org/10.1007/s10661-018-6989-4>
- National Institute of Standards and Technology – NIST. (2002). Standard Reference Materials-SRM 2709, 2710 and 2711. Retrieved February 10, 2024, from <https://www-s.nist.gov/srmors/certificates/archives/2710.pdf>.
- Oliveira, E. P., McNaughton, N. J., & Armstrong, R. (2010). Mesoarchean to Palaeoproterozoic growth of the northern segment of the Itabuna-Salvador-Curaçá orogen, São Francisco craton, Brazil. *The Geological Society*, 338, 263–286.

<https://doi.org/10.1144/SP338.13>

Oliveira, C. V., Vicente, L. C., Gama-Rodrigues, E. F., Gama-Rodrigues, A. C., Marques, J. R., & Barreto-Garcia, P. A. (2019). Carbon and nitrogen stock of Acrisols and Nitisols in South Bahia, Brazil. *Geoderma Regional*, 16, e00218. <https://doi.org/10.1016/j.geodrs.2019.e00218>

Papazotos, P., Liakopoulos, A., Kontodimos, K., & Koukoulis, A. (2024). Integrated geochemical analysis of urban and peri-urban soils: a case study of Lamia City, Greece. *Environmental Monitoring and Assessment*, 196, 1052. <https://doi.org/10.1007/s10661-024-13223-8>

Peirovi-Minaee, R., Alami, A., Moghaddam, A., & Zarei, A. (2023). Determination of concentration of metals in grapes grown in Gonabad vineyards and assessment of associated health risks. *Biological Trace Element Research*, 201, 3541–3552. (2023). <https://doi.org/10.1007/s12011-022-03428-8>

Preston, W., Nascimento, C. W. A., Biondi, C. M., Souza Junior, V. S., Silva, W. R., & Ferreira, H. A. (2014). Valores de referência de qualidade para metais pesados em solos do Rio Grande do Norte. *Revista Brasileira de Ciência do Solo*, 38, 1028–1037. <https://doi.org/10.1590/S0100-06832014000300035>

R Core Team, 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Retrieved February 01, 2024, from <https://www.R-project.org/>.

Ranta, P., Blom, T., Niemelä, J., Joensuu, E., & Siittonen M. (1998). The fragmented Atlantic Forest of Brazil: size, shape, and distribution of forest fragments. *Biodiversity and Conservation*, 7, 385-403. <https://doi.org/10.1023/A:1008885813543>

Raij, B., Andrade, J. C., Cantarella, H., & Quaggio, J. A. (2001). *Análise química para avaliação da fertilidade de solos tropicais*. IAC. Reimann, C., & Garrett, R. G. (2005). Geochemical background—Concept and reality. *Science of the Total Environment*, 350(1-3), 12-27. <https://doi.org/10.1016/j.scitotenv.2005.01.047>

Reimann, C., & Caritat, P. (2017). Establishing geochemical background variation and threshold values for 59 elements in Australian surface soil. *Science of The Total Environment*, 578, 633–648. <https://doi.org/10.1016/j.scitotenv.2016.11.010>

Resende, A. F., Gavioli, F. R., Chaves, R. B., Metzger, J. P., Pinto, L. F. G., Piffer, P. R., Krainovic, P. M., Fuza, M. S., Rodrigues, R. R., Pinho, M., Almeida, C. T., Almeida, D. R. A., Molin, P. G., Silva, T. S. F., & Brancalion, P. H. S. (2024). How to enhance Atlantic Forest protection? Dealing with the shortcomings of successional stages classification. *Perspectives in Ecology and Conservation*, 22, 101-111. <https://doi.org/10.1016/j.pecon.2024.04.002>

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., & Hirota, M. M., 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation*, 142, 1141-1153. <https://doi.org/10.1016/j.biocon.2009.02.021>

Schmidt, M., Jochheim, H., Kersebaum, K. C., Lischeid, G., & Nendel, C. (2017). Microclimate, carbon and nitrogen gradients in transition zones of fragmented landscapes –Review. *Agricultural and Forest Meteorology*, 232, 659–671. <https://doi.org/10.1016/j.agrformet.2016.10.022>

Silva, C. F., Pereira, M. G., Miguel, D. L., Feitora, J. C. F., Loss, A., Menezes, C. E. G., & Silva, E. M. R. (2012). Total organic carbon, microbial biomass and soil enzyme activity areas of agriculture, forestry and grassland in the middle Valley of Paraíba do Sul River (RJ). *Revista Brasileira de Ciência do Solo*, 36, 1680–1689. <https://doi.org/10.1590/S0100-06832012000600002>

Silva, Y. J. A. B., Nascimento, C. W. A., & Biondi, C. M. (2014). Comparison of USEPA digestion methods to heavy metals in soil samples. *Environmental Monitoring and Assessment*, 186, 47–53. <https://doi.org/10.1007/s10661-013-3354-5>

Silva, W. C., Cassol, P. C., Nicoloso, R. S., Mumbach, G. L., Dall'Orsoletta, D. J., Grando, D. L., & Gatiboni, L. C. (2023). Establishing environmental soil phosphorus thresholds to mitigate its transfer to water bodies in Mato Grosso State, Brazil. *Revista Brasileira de Ciência do Solo*, 47, e0230049. <https://doi.org/10.36783/18069657rbc20230049>

Silva, T. M., Garcia, K. G. V., Silva, Y. J. A. B., Teixeira Filho, C. D., Cavalcante, R. M., Boechat, C. L., Pereira, A. P. A., & Escobar, M. E. O. (2024a). Contamination risk by heavy metals and enzymatic stoichiometry in agricultural soils under intense use of pesticides. *Environmental Monitoring and Assessment*, 196, 805. <https://doi.org/10.1007/s10661-024-12965-9>

Silva, F. L., Martins e Silva, M. H., Veiga, J. B., Silva, A. C. S., Carvalho, M. A. C., Weber, O. L. S., Eguchi, E. S., López-Alonso, M., Oliveira-Júnior, E. S., Guilherme, L. R. G., & Pierangeli, M. A. P. (2024b). Assessing background levels of trace elements in soils of Mato Grosso (Brazil) for environmental and food security. *Catena*, 244, 108267. <https://doi.org/10.1016/j.catena.2024.108267>

Soil Survey Staff., 2014. *Keys to Soil Taxonomy*. twelfth ed. USDA - Natural Resources Conservation Service.

Souza, D. F. M., Oliveira, E. P., Amaral, W. S., & Baldim, M. R. (2020). The Itabuna-Salvador-Curaçá Orogen revisited, São Francisco Craton, Brazil: New zircon U–Pb ages and Hf data support evolution from archean continental arc to paleoproterozoic crustal reworking during block collision. *Journal of South American Earth Sciences*, 104, 102826. <https://doi.org/10.1016/j.jsames.2020.102826>

SOSMA, 2016. *Atlas dos remanescentes florestais. Mapas*. Retrieved April 25, 2024, from <https://www.sosma.org.br/iniciativas/atlas-da-mata-atlantica>.

SOSMA, 2021. *Atlas dos remanescentes florestais da Mata Atlântica, 2019-2020*. Retrieved April 25, 2024, <http://mapas.sosma.org.br/>.

Štrbac, S., Randelović, D., Gajica, G., Hukić, E., Stojadinović, S., Veselinović, G., & Kašanin-Grubin, M. (2022). Spatial distribution and identification of the source of heavy

metals in soils of European montane beech forests. *Chemosphere*, 309, 136662. <https://doi.org/10.1016/j.chemosphere.2022.136662>

Taghavi, M., Bakhshi, K., Zarei, A., & Gholizadeh, A. (2024). Soil pollution indices and health risk assessment of metal(loid)s in the agricultural soil of pistachio orchards. *Scientific Reports*, 14, 8971. <https://doi.org/10.1038/s41598-024-59450-4>

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). *Manual de métodos de análise de solo*. third ed. Embrapa.

United States Environmental Protection Agency – USEPA, 2007. *Method 3051A – Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils*. Retrieved February 13, 2024, from <https://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3051a.pdf>.

Weng, C., Jia, S., Wang, D., & Ma, W. (2024). Pollution characteristics of heavy metal(loid)s in soil in China: Implications for health risk assessment and temporal trend analysis. *Environmental Chemistry and Ecotoxicology*, 6, 248-258. <https://doi.org/10.1016/j.eneco.2024.07.007>.

**ARTICLE III**

Trace-element concentrations and ecological risk potential in soils under “cabruca” agroforestry system in the cacao-growing region of Bahia, northeastern Brazil\*

---

\* **Situation:** Not submitted

## **Trace element concentrations and ecological risk potential in soils under “cabruca” agroforestry system in the cacao-growing region of Bahia, northeastern Brazil**

Tatiana Reis dos Santos Bastos <sup>a</sup>, João Carlos Medeiros <sup>b</sup>, Kaique Mesquita Cardoso <sup>a, c</sup>, Clístenes Williams Araújo do Nascimento <sup>d</sup>, Paula Nascimento Alves <sup>e</sup>, Pâmalla Graziely Carvalho Morais <sup>e</sup>, Glécio Machado Siqueira <sup>f</sup>, Maria Eugênia Ortiz Escobar <sup>g</sup>, Cácio Luiz Boechat <sup>a, e, \*</sup>

<sup>a</sup> State University of Southwest Bahia (UESB), Graduate Program in Agronomy, Vitória da Conquista, Bahia, 45083-900, Brazil. 2021s0001@uesb.edu.br, analuisa.leite.pereira@gmail.com.

<sup>b</sup> Federal University of Southern Bahia (UFSB), Itabuna, Bahia, 45600-923, Brazil. joao.medeiros@ufsb.edu.br, geison.santos@gfe.edu.ufsb.br

<sup>c</sup> Federal Institute of Education, Science and Technology of Northern Minas Gerais (IFNMG), Araçuaí, Minas Gerais, Brazil, 39600-000. kaique.cardoso@ifnmg.edu.br.

<sup>d</sup> Federal Rural University of Pernambuco (UFRPE), Recife, Pernambuco, 52171-900, Brazil. clistenes.nascimento@ufrpe.br.

<sup>e</sup> Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas, Rodovia Bom Jesus - Viana, s/n, Planalto Horizonte, Bom Jesus, Piauí, 64900-000, Brazil. paula.alves@ufpi.edu.br, pamalla\_grazielly@ufpi.edu.br.

<sup>f</sup> Federal University of Maranhão (UFMA), São Luís, Maranhão, 65080-805, Brazil. glécio.siqueira@ufma.br.

<sup>g</sup> Federal University of Ceará (UFC), Fortaleza, Ceará, 60455760, Brazil. mariaeugenia@ufc.br.

\* Corresponding author at: Campus Prof<sup>a</sup> Cinobelina Elvas, Federal University of Piauí, Rodovia Bom Jesus-Viana, km 01, S/N, Planalto Horizonte, Bom Jesus, Piauí 64900-000, Brazil.

E-mail address: cacioboechat@ufpi.edu.br (C.L. Boechat). Cellphone: +55 89 981334699

### **Highlights:**

Spatial distribution of trace elements is governed by the geological context.

The cacao-growing region falls within a range of minimal to moderate pollution. Cadmium (Cd) stands out due to its individual ecological risk in cultivated areas.

The findings can inform environmental agencies and support decision-making processes.

## ABSTRACT

Trace element contamination in agricultural soils is a growing concern, particularly in tropical regions. Although considered sustainable, agroforestry systems such as cacao-cabruca system have rarely been assessed for these risks. The study area, located in the cacao-producing region of Bahia, Brazil, is a major hub of high cacao productivity. This context underscores the need to monitor trace elements due to their persistence and toxicity in soils. The objectives of this study were: (1) to quantify trace element concentrations and their vertical distribution in soils under cacao-cabruca systems; (2) to assess contamination levels using geochemical and ecological indices: Igeo (geoaccumulation index), CF (contamination factor), EF (enrichment factor), ERi (individual ecological risk index), and PERI (potential ecological risk index); and (3) to evaluate potential anthropogenic impacts. The study focused on trace elements (Cd, Co, Cr, Cu, Mo, Ni, Pb, V, Zn, Fe) in cacao-cabruca agroforestry systems across 10 municipalities. A total of 50 composite soil samples were collected at depths of 0–5, 5–10, 10–20, 20–40, and 40–60 cm. Samples were analyzed for fertility and texture, with trace elements extracted following EPA Method 3051A and quantified via ICP- OES. Higher concentrations of Co, Cu, Mo, Pb, and Zn were observed compared to local soil quality reference values, while Cd, Cr, Ni, V, and Fe remained below thresholds. Igeo showed 88% of surface samples (0–5 cm) classified as unpolluted to moderately polluted. Mo showed elevated contamination factors at all depths. EF values were approximately zero, indicating no anthropogenic enrichment. ERi showed moderate ecological risk only for Cd. All PERI values indicated low ecological risk (PERI < 150). The findings underscore the need for targeted monitoring of cacao production systems to mitigate contamination risks. Although overall ecological risk was low, elevated levels of Mo and Cd highlight the need for targeted monitoring, even in low-input systems such as cacao-cabruca. The combined use of geochemical and ecological indices (Igeo, CF, EF, ERi, PERI) proved effective in identifying subtle contamination patterns and potential anthropogenic influence. These results underscore the importance of multi-index approaches for assessing soil quality and guiding sustainable management in tropical agroforestry systems.

**Keywords:** Environmental monitoring, Soil health, Heavy metals, Environmental risk, Soil contamination, *Theobroma cacao*.

## 5.1 INTRODUCTION

Soil serves as the primary sink for contaminants in terrestrial ecosystems, functioning serving as a natural reservoir for pollutants originating from both natural and anthropogenic sources (Lian et al., 2019). Trace elements, including metals and metalloids, are naturally occurring components of the Earth's crust and are widely distributed across soils. Although the mineralogical composition of parent materials strongly influences the natural background concentrations of trace elements, several anthropogenic activities have significantly increased their levels in soils (Cai et al., 2019), often exceeding natural reference values (Bolan et al., 2013; Hou et al., 2020; Alves et al., 2024; Cardoso et al., 2024).

Soil contamination by trace elements has increasingly been recognized as a major environmental concern (Mansour et al., 2014) due to their toxicity, persistence, non-biodegradability, and potential for bioaccumulation (Islam et al., 2015). The accumulation of metals in agricultural soils can compromise crop productivity and quality (Chen et al., 2023; Ahmad et al., 2023), in addition to degrading overall ecological soil quality (Sarwar et al., 2017; Rajendran et al., 2022). These contaminants can be transferred from soil to plants and, subsequently, to the food chain, posing risks to human health, especially among vulnerable populations (Lian et al., 2022; Ejaz et al., 2023).

The cacao-producing region of Bahia state, Brazil, internationally recognized for the traditional "Cabraça" agroforestry system, has regained global prominence in cacao production. With annual cacao bean production of over 110 thousand tons, the region leads Brazil's national production, reaching a national record of USD 434 million in exports in 2024 (MAPA, 2024). This expansion highlights the urgent need for sustainable soil management strategies, including monitoring trace elements and assessing their potential effects (Huang et al., 2016) on a regional scale while considering the inherent risks to human and environmental health (Saha et al., 2024). Previous studies have shown that tree species commonly used in agroforestry systems may reduce the mobility of trace elements in soil, limiting their leaching to deeper layers or uptake by crops (Ali et al., 2013; Khan et al., 2015; Souza et al., 2019). However, the effectiveness of such systems may be limited in the Atlantic Forest remnants of Bahia's cacao region, which have undergone severe fragmentation due to historical agricultural expansion. In this context, assessing the risks associated with trace elements in soil becomes particularly relevant to support regional contamination monitoring and inform public

policies aimed at environmental protection and health risk mitigation (Liu et al., 2020).

To accurately assess soil contamination, a key concept is the soil quality reference value (CONAMA, 2009; Rutkowski et al., 2020). These reference values, often used as comparative standards (Kabata-Pendias, 2010), are widely employed in environmental risk assessments related to trace elements in soils (Zhang et al., 2017; Li et al., 2018). Ecological risk indices based on soil parameters and trace element concentrations are also employed to quantify soil pollution levels (Barkett & Akün, 2018), providing a systematic framework for soil quality assessment and identifying potential contamination sources (Alves et al., 2024).

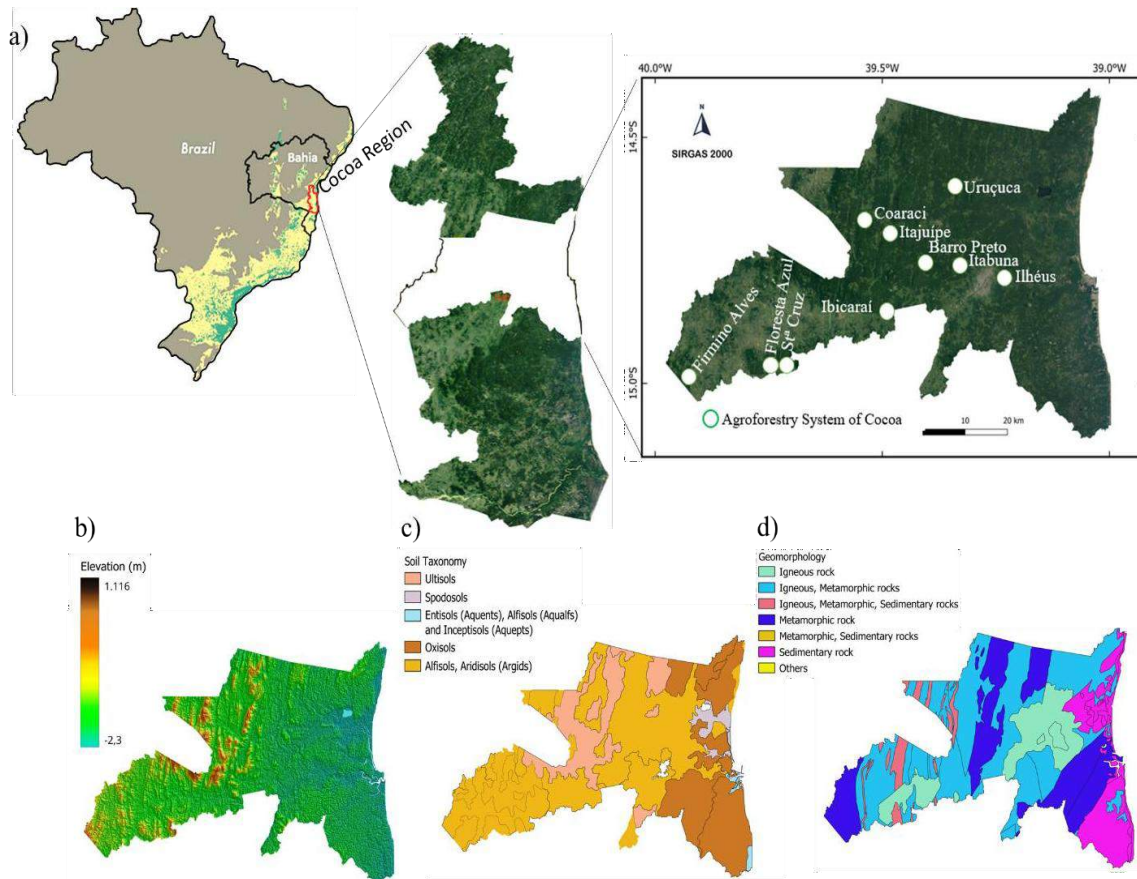
The present study significantly expands on this approach by integrating data from 10 municipalities and multiple factors: soil depth and a range of contamination indices, including the Geoaccumulation Index (Müller, 1969), Contamination Factor (Hakanson, 1980), Enrichment Factor (Sutherland, 2000), Individual Ecological Risk (Hakanson, 1980), and Potential Ecological Risk (Wang et al., 2015) for each soil layer. This holistic framework enhances the understanding of contaminant dynamics within the region's soils.

Although the cacao-cabruca agroforestry system is known for its sustainability and low-impact agricultural practices, we hypothesize that specific local practices of inadequate management may contribute metals in these areas promoting the increase in concentrations of trace elements (Cd, Co, Cr, Cu, Mo, Ni, Pb, V, Zn, Fe) over time. Therefore, the aims of this study were: (1) to quantify the concentration and spatial distribution of trace elements in the soil profile (0–5, 5–10, 10–20, 20–40, 40–60 cm); (2) to assess the degree of soil pollution using various indices based on soil quality reference values; and (3) to evaluate the effectiveness of ecological risk indices ( $I_{geo}$ , CF, EF,  $ER_i$ , and PERI) in identifying the degree of anthropogenic impact in the cacao-growing soils of Bahia.

## **5.2 MATERIAL AND METHODS**

### **5.2.1 Study area**

The cacao-growing region of Bahia is located within the Atlantic Forest biome in northeastern Brazil, covering 41 municipalities between longitudes 38°30'W and 40°00'W and latitudes 13°30'S and 16°00'S, with elevations ranging from 2 to 1,116 meters above sea level (Figs. 1a and 1b).



**Figure 1** - Location of the study areas (a), altitude (b), soil classes (c), and geology (d) of soils under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia, Northeastern Brazil.

The region covers approximately 21,000 km<sup>2</sup> and is Brazil's largest cacao-producing area, followed by the state of Pará (IBGE, 2024), with municipalities such as Ilhéus being particularly significant (Fig. 1a). Ilhéus and its surrounding municipalities are characterized by favorable edaphoclimatic conditions for sustainable cacao cultivation, including average annual temperatures between 23 and 26 °C and a humid tropical climate according to the Köppen-Geiger classification (Köppen, 1948). This climatic stability, combined with diverse soil types (Fig. 1c), predominantly Oxisols, Ultisols, Alfisols, and Spodosols (Soil Survey Staff, 2014), and underlying lithology (Fig. 1d) from the São Francisco Craton shaped by the Itabuna-Salvador-Curaçá orogeny (Oliveira et al., 2010), together with remnants of the Atlantic Forest, highlights the ecological and agricultural significance of the region.

## 5.2.2 Experimental design, sampling, and soil analysis

Ten municipalities within a 100-km radius of Ilhéus were selected to represent a geographic gradient of the cacao-cabruca agroforestry system and include both small and large production properties (5–100 hectares). Collectively, these municipalities cover an area of 4,287.88 km<sup>2</sup> and have a population of 495,506 inhabitants, representing approximately 50% of the cacao region's total population (IBGE, 2024).

Each municipality was treated as an experimental unit, with soil sampling conducted on farms practicing the cabruca agroforestry system. Cacao trees, typically older than 50 years, were interspersed with native tree species under a thinned forest canopy. Sampling plots were measured 50 m × 50 m. All sampled farms avoided chemical fertilizers, instead applying lime for pH correction, returning organic residues from fruit pods beneath the canopy, and performing annual pruning and manual harvesting.

Composite soil samples were collected at depths of 0–5, 5–10, 10–20, 20–40, and 40–60 cm. Each composite consisted of five sub-samples taken at the cardinal points (north, south, east, west, and center) using a stainless-steel Dutch auger. The samples were homogenized by depth, resulting in a total of 50 samples. Geographic coordinates of each site were recorded using a Garmin GPS receiver with UTM projection and the SIRGAS 2000 datum.

### **5.2.3 Chemical and particle size analysis**

Soil samples were air-dried, gently disaggregated, homogenized, and passed through a 2.0 mm stainless-steel mesh. Chemical and particle-size analyses followed the methodology recommended by Raij et al. (2001). Soil pH was determined in a 1:2.5 (w/v) soil-to-water suspension. Available phosphorus (P) and potassium (K) were extracted using Mehlich-1 solution (HCl + H<sub>2</sub>SO<sub>4</sub>). Exchangeable calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), and aluminum (Al<sup>3+</sup>) were extracted using 1 mol L<sup>-1</sup> KCl solution and determined by titration. Potential acidity (H<sup>+</sup> + Al<sup>3+</sup>) was estimated using buffered calcium acetate solution at pH 7.0. These results were used to calculate cationexchange capacity (CEC), base saturation (V%), and aluminum saturation (m%).

Total organic carbon (TOC) and total nitrogen (TN) were quantified simultaneously by elemental analysis using a CHN elemental analyzer, following the protocol described by Baldock et al. (2013). Particle-size analysis was performed using the pipette method (Teixeira et al., 2017), which involved dispersion in water, agitation, controlled sedimentation, and fraction separation with calibrated pipettes.

#### 5.2.4 Determination of trace elements in soil

Soil samples were ground in an agate mortar and sieved through a 0.15 mm stainless-steel mesh (ABNT no. 100). One gram of each sample was transferred to Teflon<sup>®</sup> digestion tubes and treated with 12 mL of aqua regia (HNO<sub>3</sub>: HCl at a 3:1 v/v ratio, analytical grade, Merck PA) for trace element extraction (Cd, Co, Cr, Cu, Mo, Ni, Pb, V, Zn, and Fe). The digestion followed USEPA Method 3051A (USEPA, 2007), recommended by the Brazilian National Environmental Council (CONAMA, 2009), using a closed-vessel microwave system (Ethos Easy, Milestone) at 175 °C for 17 minutes, followed by 4 minutes and 30 seconds for cooling.

After digestion, extracts were filtered with slow-filter paper (Macherey- Nagel<sup>®</sup>) and transferred to certified 25 mL volumetric flasks (NBR ISO/IEC), with volumes completed using ultrapure water (Milli-Q, 18.2 MΩ·cm). Trace element concentrations in the extracts were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer 7000 DV) equipped with an autosampler. Calibration curves were prepared using multielement standards (1000 mg L<sup>-1</sup>, Titrisol<sup>®</sup>, Merck/Sigma-Aldrich) within the following ranges: Cd (0.02–2), Co (0.05–5), Cr (0.01–10), Cu (0.01–10), Fe (0.5–1000), Mo (0.05–5), Ni (0.02–10), Pb (0.02–5), V (0.01–10), and Zn (0.01–10) mg L<sup>-1</sup>. Analytical quality control included reagent blanks and a certified reference material (SRM 2709 – San Joaquin Soil, NIST, 2002), consistent with validation in previous studies (Boechat et al., 2020; Cardoso et al., 2023; Alves et al., 2024).

#### 5.2.5 Ecological Risk Indicators

To assess ecological risk, soil trace element concentrations were compared with regional soil quality reference values (75th percentile), arranged in increasing order (mg kg<sup>-1</sup>): Mo (0.13) < Cd (3.47) < Pb (7.81) < Ni (11.45) < Co (12.50) < Cu (16.99) < Zn (24.02) < V (50.12) < Cr (54.24) < Fe (32,740) (Bastos et al., 2025). The following indices were used:

##### 5.2.5.1 Geoaccumulation Index

The geoaccumulation index ( $I_{geo}$ ) assesses soil pollution levels based on the ratio

between measured trace element concentrations and corresponding background values, as proposed by Müller (1969) and adapted by Zhao et al. (2012). It is calculated by the Equation 1:

$$I_{geo} = \log_2 \frac{C_i}{1.5B_n} \quad (1)$$

Where:

$I_{geo}$  = Geoaccumulation index

$C_i$  = Measured concentration of the element in the soil ( $\text{mg kg}^{-1}$ )

$B_n$  = Background/reference value of the element ( $\text{mg kg}^{-1}$ )

1.5 = Correction factor, used to minimize possible natural variations due to lithological differences

#### 5.2.5.2 Contamination Factor

The contamination factor (CF) expresses the ratio between the concentration of a trace element in the sample and its natural background value, without normalization to a conservative element (Hakanson, 1980; Fei et al., 2019). It is given by Equation 2:

$$CF = \frac{C_i}{C_b} \quad (2)$$

Where:

$C_i$ : Measured concentration of the element ( $\text{mg kg}^{-1}$ )

$C_b$  = Background value of the element ( $\text{mg kg}^{-1}$ )

#### 5.2.5.3 Enrichment Factor

The enrichment factor (EF) compares the concentration of a trace element in the sample with its reference value, normalized to a conservative reference element, in this study iron (Fe), due to its low mobility and stable geochemical behavior (Sutherland, 2000). It is calculated by Equation 3:

$$EF = \frac{C_i/C_r}{C_{bi}/C_{br}} \quad (3)$$

Where:

- $C_i$ : Concentration of the element in the sample ( $\text{mg kg}^{-1}$ )  
 $C_r$ : Concentration of reference element Fe in the sample ( $\text{g kg}^{-1}$ )  
 $C_{b_i}$ : Background value of the element ( $\text{mg kg}^{-1}$ )  
 $C_{b_r}$ : Background value of the reference element Fe ( $\text{g kg}^{-1}$ )

#### 5.2.5.4 Individual ecological risk index

The individual ecological risk index ( $ER_i$ ) evaluates the potential ecological risk of each element by combining its toxicity with its contamination factor (Hakanson, 1980; Wang et al., 2015). It is expressed by Equation 4:

$$E_r^i = T^i \times CF \quad (4)$$

Where:

$E_r^i$ : Potential ecological risk factor of each metal

$T^i$ : Toxic response factor for a given metal (Cd = 30, Co = 5, Cr = 2, Cu = 5, Mo = 0,46, Ni = 6, Pb = 5, V = 2, Zn = 1, and Fe = 32,73).

CF: Contamination factor

#### 5.2.5.5 Potential ecological risk index

The potential ecological risk index (PERI) represents the cumulative soil risk, calculated as the sum of the individual ecological risks ( $ER_i$ ) (Wang et al., 2015; Fei et al., 2019), according to Equation 5.

$$PERI = \sum_{i=1}^n E_r^i \quad (5)$$

Where:

$n$  = Number of trace elements assessed

$E_r^i$  = Individual ecological risk index of each element

To interpret the results, risk categories were adopted based on index values Table 1

presents the classification ranges used in this study for  $I_{geo}$ , CF, EF,  $ER_i$ , and PERI.

**Table 1.** Categories of the geoaccumulation index ( $I_{geo}$ ), contamination factor (CF), enrichment factor (EF), individual ecological risk ( $ER_i$ ) and potential ecological risk index (PERI).

$I_{geo}$	Categories	CF	Categories	EF	Categories	$ER_i$	Categories	PERI	Categories
$I_{geo} \leq 0$	Pollution-free	$\leq 1$	Pollution free	$\leq 1$	No enrichment	$< 40$	Low risk	$< 150$	Low risk
$0 < I_{geo} \leq 1$	Unpolluted	1–2	Lightweight	1–2	Minimal	40–80	Moderate	150–300	Moderate
$1 < I_{geo} \leq 2$	Moderately polluted	2–3	Lightweight	2–5	Moderate	80–160	Considerable	300–600	Considerable
$2 < I_{geo} \leq 3$	Moderately to very polluted	3–5	Moderate	5–20	Significant	160–320	High	$> 600$	High risk
$3 < I_{geo} \leq 4$	Very polluted	$> 5$	High pollution	20–40	Very high	$> 320$	Very high	–	–
$4 < I_{geo} \leq 5$	Very to extremely polluted	–	–	$> 40$	Extremely high	–	–	–	–
$I_{geo} > 5$	Highly polluted	–	–	–	–	–	–	–	–

### 5.2.6 Statistical analysis

Normality of the data was evaluated using the Shapiro-Wilk test, while homogeneity of variances across treatments and soil depths was assessed with Levene’s test. Since the assumptions of normality and homoscedasticity were not met, even after data transformation, non-parametric statistical methods were applied. Specifically, the Kruskal-Wallis H test was used, adopting a significance level set at 5% ( $p < 0.05$ ).

Descriptive statistics—including minimum, maximum, mean, median, standard deviation, and coefficient of variation—were computed to summarize the fundamental characteristics of trace element concentrations. Furthermore, Spearman’s rank correlation analysis was performed to examine relationships between trace elements and environmental variables such as climate, geology, soil class, elevation, clay content, organic carbon, aluminum, and pH.

All statistical analyses were conducted using Python programming language (Python Software Foundation, 2024), version 3.11.

## 5.3. RESULTS AND DISCUSSION

### 5.3.1 Soil attributes

Soils under the cacao-cabruca agroforestry system exhibited considerable heterogeneity in the evaluated attributes (Table 2). Soil pH ranged widely, from a minimum of 4.37, indicating high acidity, to a maximum of 6.99, approaching neutrality. The median pH was 5.62, with an overall mean of 5.76, characterizing the soils as acidic to slightly acidic. Such conditions may limit nutrient availability and increase the risk of metal toxicity due to elevated  $H^+$  ion concentrations as pH decreases (Zhu & Shen, 2024).

Available phosphorus content showed substantial variability (6.45 to 87.99  $mg\ dm^{-3}$ ), likely influenced by aluminum saturation levels ranging from 0.0% to 63.66%. As reported by Wang and Liao (2023), aluminum toxicity and phosphorus deficiency commonly coexist in acidic soils, with Al-P precipitate formation contributing to the low phosphorus bioavailability. Total organic carbon (TOC) and total nitrogen (TN) also varied widely, from 2.3 to 26.7  $g\ kg^{-1}$  and 0.3 to 2.2  $g\ kg^{-1}$ , respectively. According to the USDA textural classification (2014), soils were predominantly sandy loam, with mean proportions of 58.8% sand, 21.04% clay, and 20.74% silt—typical of tropical agricultural regions.

**Table 2.** Soil attributes in the agroforestry system in the cacao-growing region of Bahia, northeastern Brazil (n = 50).

Variable	Min	Median	Mean	Max	SD	Skewness	Kurtosis
pH H <sub>2</sub> O (1:2,5)	4.37	5.62	5.76	6.99	0.94	-1.87	7.72
Al ( $cmol_c\ dm^{-3}$ )	0.00	0.00	0.62	12.50	1.90	5.25	29.15
H+Al ( $cmol_c\ dm^{-3}$ )	1.01	3.50	3.93	11.08	2.59	0.63	-0.50
Ca ( $cmol_c\ dm^{-3}$ )	0.37	4.64	5.20	16.63	3.06	1.11	2.07
Mg ( $cmol_c\ dm^{-3}$ )	0.71	2.64	3.23	10.31	2.12	1.33	1.46
K ( $cmol_c\ dm^{-3}$ )	0.00	0.04	0.06	0.31	0.06	1.88	3.47
P ( $mg\ dm^{-3}$ )	6.55	19.14	24.15	87.99	17.44	1.54	2.36
SB ( $cmol_c\ dm^{-3}$ )	1.64	7.17	8.49	22.63	4.61	1.02	0.79
CEC ( $cmol_c\ dm^{-3}$ )	4.02	11.78	12.42	25.88	5.55	0.73	-0.23
V (%)	20.49	69.89	67.82	95.18	17.30	-0.61	-0.12
m (%)	0.00	0.00	6.16	63.66	13.15	2.98	8.95
TOC ( $g\ kg^{-1}$ )	23.00	10.2	11.3	26.7	0.61	0.59	-0.36
TN ( $g\ kg^{-1}$ )	0.30	0.90	1.0	2.02	0.05	0.63	-0.48
sand (%)	231.58	633.61	582.25	788.77	154.24	-0.39	-1.22

clay (%)	100.59	177.69	210.38	554.59	107.08	1.30	0.96
silt (%)	67.9	193.0	207.37	456.46	89.62	0.80	0.34

TN = total nitrogen SD = Standard Deviation.

### 5.3.2 Trace element concentrations in soils under cacao cultivation

Variations were observed in the mean concentrations of trace elements (Cd, Co, Cr, Cu, Mo, Ni, Pb, V, Zn, and Fe) across the study areas located in the cacao- growing region of Bahia (Table 3). The average concentrations decreased in the following order: Fe (21,497.89 mg kg<sup>-1</sup>) > Zn (30.63 mg kg<sup>-1</sup>) > V (27.87 mg kg<sup>-1</sup>) > Cr (23.79 mg kg<sup>-1</sup>) > Co (16.06 mg kg<sup>-1</sup>) > Cu (14.72 mg kg<sup>-1</sup>) > Pb (8.60 mg kg<sup>-1</sup>) > Ni (7.54 mg kg<sup>-1</sup>) > Cd (1.98 mg kg<sup>-1</sup>) > Mo (0.82 mg kg<sup>-1</sup>) (Table 3, Fig. 2). These results indicate that trace element concentrations varied significantly across samples, reflecting the influence of local soil properties and potential anthropogenic inputs.

**Table 3.** Descriptive statistics of trace elements in soils affected by human activities in the cacao-growing region of Bahia, northeastern Brazil (n = 50).

Trace elements	Cd	Co	Cr	Cu	Mo	Ni	Pb	V	Zn	Fe
mg kg <sup>-1</sup>										
Min	0.0	1.74	7.29	4.34	0.0	1.14	3.64	7.99	8.08	3,639.22
Max	6.95	68.64	83.04	49.84	2.19	33.69	22.84	60.65	58.33	57,399.84
Mean	1.98	16.06	23.79	14.72	0.82	7.54	8.60	27.87	30.63	21,497.89
Median	1.31	13.79	22.29	12.64	0.75	5.79	7.68	24.84	29.98	13,314.41
SD	1.83	15.97	14.18	8.37	0.55	7.68	4.60	15.52	16.01	17,140.98
CV (%)	92.42	99.43	59.63	56.86	67.07	101.85	53.48	55.68	52.26	79.73
Skewness	1.30	1.98	1.71	1.80	0.39	2.24	1.44	0.64	0.15	0.76
Kurtosis	0.56	3.82	4.97	5.25	-0.36	4.66	1.62	-0.70	-1.53	-1.10
X2(4)	36.1	26.5	17.38	20.72	29.93	8.78	19.99	31	33.48	30.78
Significant	***	***	**	***	***	ns	***	***	***	***
p-value	2,76E+07	0.000	0.001	0.000	5,05E+08	0.066	0.000	3,06E+08	9,53E+07	3,40E+08

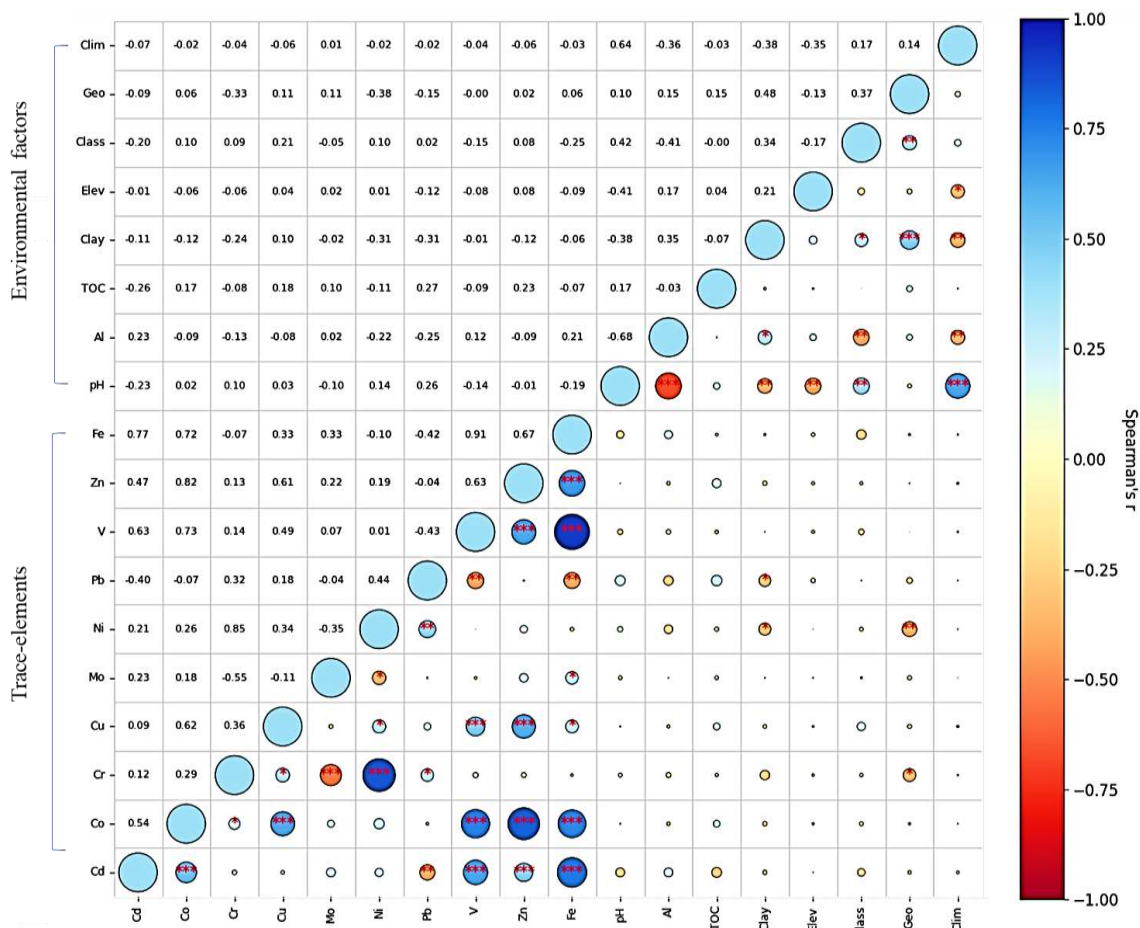
The coefficient of variation (CV) reflects differences in trace element concentrations among sampling points in the soil (Gallardo & Paramá, 2007). The elements showed a decreasing order of variability: Ni (101.85%) > Co (99.43%) > Cd (92.42%) > Fe (79.73%) > Mo (67.07%) > Cr (59.63%) > Cu (56.86%) > V (55.68%) > Pb (53.48%) > Zn (52.26%). Higher CV values indicate greater impacts from anthropogenic activities, whereas lower CV values suggest a stronger influence of natural

factors (Baltas et al., 2020). CV values greater than 90% for Ni, Co, and Cd suggest these elements are more likely to be influenced not only by parent material but also by human activities, as reflected in localized concentration peaks.

Skewness analysis revealed considerable variation among elements. Several metals exhibited strong positive skewness (right-skewed distributions), with extremely high values relative to the mean in approximately 60% of cases. This pattern may indicate localized accumulation, variations in soil mineralogy, or anthropogenic interference. Conversely, elements such as Zn ( $0.15 \text{ mg kg}^{-1}$ ), Mo ( $0.39 \text{ mg kg}^{-1}$ ), V ( $0.64 \text{ mg kg}^{-1}$ ), and Fe ( $0.76 \text{ mg kg}^{-1}$ ) exhibited skewness values close to zero, suggesting relatively symmetrical distributions and greater homogeneity in the dataset. Kurtosis values for trace elements ranged from  $-1.53$  to  $5.28 \text{ mg kg}^{-1}$ , indicating distributions from platykurtic (low frequency of outliers) to leptokurtic (high frequency of outliers). These differences reflect distinct distribution patterns of trace element concentrations in the soil.

### **5.3.3 Relationship between environmental factors and trace element**

Trace elements, due to their low natural abundance, are highly sensitive to environmental factors such as climate, geology, soil class, elevation, clay content, total organic carbon, available aluminum, and pH. These factors directly affect their physicochemical speciation and behavior in soil (N'Guessan et al., 2009). To evaluate the relationships between environmental variables and trace element concentrations in the cacao-growing soils of Bahia, Spearman's correlation analysis was applied and the results are shown in Fig. 2.



**Figure 2** - Spearman's correlation analysis between environmental factors and trace elements concentrations in soils from the cacao-growing region of Bahia, northeastern Brazil. Elev = Elevation; Class = Soil class; Geo = Geology; Clim = Climate. (\*\*\*)  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$  indicate statistically significant correlations).

Soil pH showed a moderate negative correlation with Cd ( $-0.23$ ) and Fe ( $-0.19$ ), and a moderate positive correlation with Pb ( $0.26$ ), while correlations with other trace elements were statistically non-significant. These results indicate that in more alkaline soils, the mobility and bioavailability of Cd and Fe tend to decrease. In contrast, Pb behavior is more complex and may be influenced by additional environmental or soil-specific factors. For the remaining elements, soil pH did not significantly influence their concentrations, which may be related to the chemical form in which the metals are present—likely in the residual (non-bioavailable) fraction—or to the stronger influence of other environmental drivers.

Aluminum exhibited a moderate negative correlation with Ni ( $-0.22$ ) and Pb ( $-0.25$ ), which may reflect the competitive interaction of reactive  $Al^{3+}$  ions for sorption sites, thereby limiting Ni and Pb fixation. This trend could also indicate different geological

sources for Al and these trace elements. Conversely, Al showed moderate positive correlations with Cd (0.23) and Fe (0.21), suggesting that higher Al concentrations may enhance Cd accumulation and potentially exert a synergistic toxic effect. Additionally, Al and Fe may act as cementing agents in the formation of stable organo-mineral complexes (Šimanský & Jonczak et al., 2020).

Soil organic matter serves as an important sink for many trace elements and directly influences their availability and mobility through physical sorption, precipitation, and chelation (Huang et al., 2017). This may explain the moderate correlations observed between TOC and Cd (−0.26), Pb (0.27), and Zn (0.23). However, the negative correlation between Cd and TOC was unexpected, given the well-documented affinity of organic matter for this metal. A plausible explanation is that Cd in these soils may be of geogenic origin and therefore not directly associated with organic matter, especially if present in the residual fraction (Adriano, 2001). Ultimately, the distribution, mobility, bioavailability, and toxicity of trace elements depend not only on total concentrations but also on their chemical speciation in soil (Zovko & Romić, 2011).

A moderate negative correlation was observed between clay content and both Ni (−0.31) and Pb (−0.31), suggesting that higher clay levels reduce the availability of these metals, likely due to the retention and immobilization capacity of clay particles. Geological factors were negatively correlated with Ni (−0.38) and Cr (−0.33), consistent with the findings of Bastos et al. (2025), who reported natural concentrations of Ni and Cr below the Soil Quality Reference Values (SQRVs) in the same region, reinforcing the geogenic control over these elements.

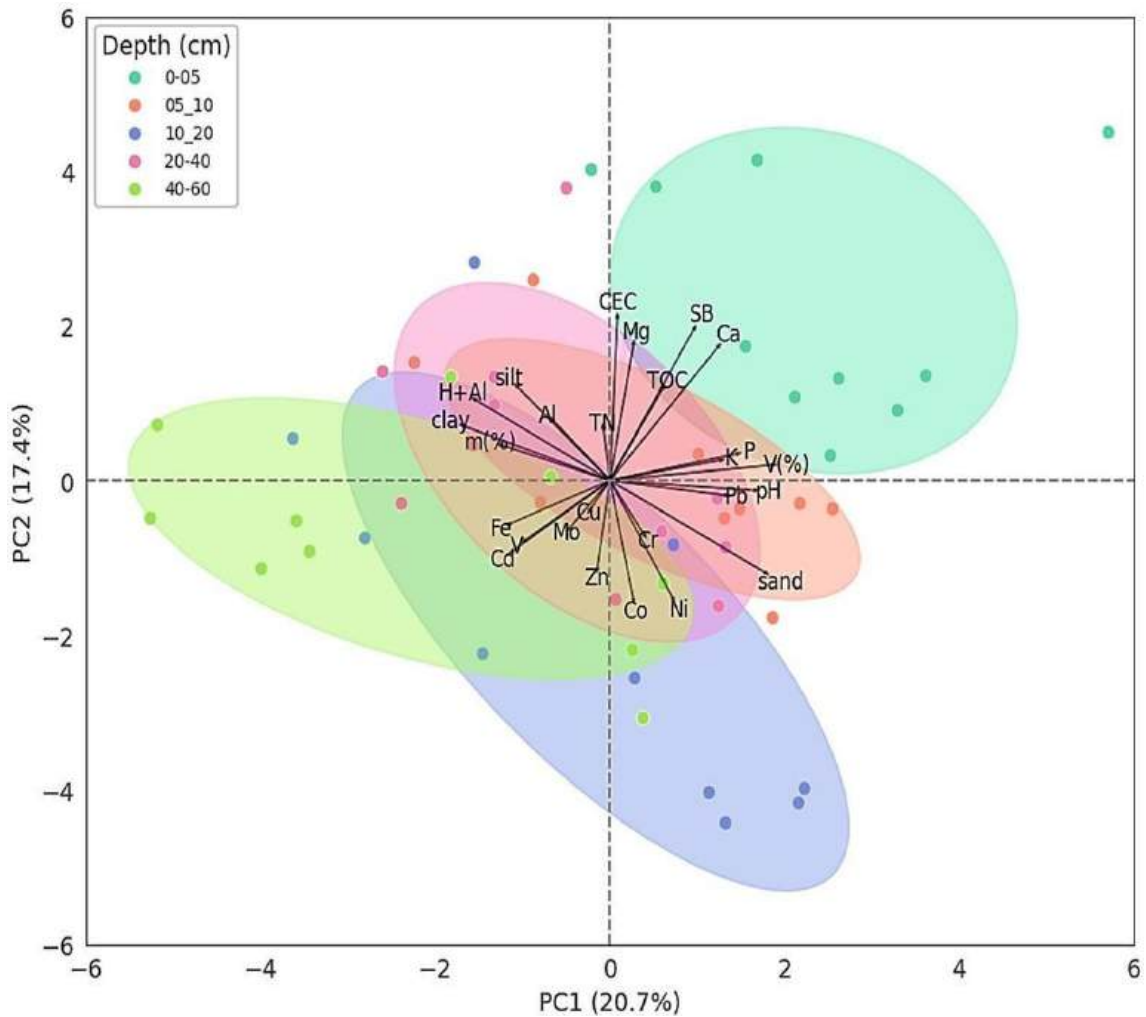
No significant correlations were detected between climate or soil class and trace metal concentrations, indicating that under the evaluated conditions, there is insufficient statistical evidence to support a strong or direct association between these variables.

Conversely, strong positive correlations were identified among several trace element pairs: Fe–V (0.91), Ni–Cr (0.85), Zn–Co (0.82), Fe–Cd (0.77), V–Co (0.73), Fe–Co (0.72), V–Cd (0.63), Co–Cu (0.62), and Co–Cd (0.54). These findings indicate that trace elements tend to co-occur due to shared geogenic sources, similar environmental behavior, or overlapping anthropogenic inputs.

#### **5.3.4 Principal Component Analysis**

Principal Component Analysis (PCA) revealed a clear influence of soil depth

on the distribution of both soil attributes and trace elements, explaining 38.1% of the total variance—an important finding considering the complexity of the environmental dataset analyzed (Fig. 3 and Table 4).



**Figure 3** - Biplot from Principal Component Analysis (PCA) of soil attributes and trace element concentrations along the soil profile. m (%) = aluminum saturation; C/N = carbon-to-nitrogen ratio; V = base saturation; SB = sum of bases; H+Al = potential acidity; TOC = total organic carbon; TN = total nitrogen. Colored ellipses represent the dispersion of sample points within each soil layer across the study areas located in the cacao-growing region of Bahia.

**Table 4** - Principal Component Analysis (PCA) of soil fertility attributes, particle-size distribution, and trace element concentrations across study areas within the cacao-growing region of Bahia, northeastern Brazil.

Variable	PC1	PC2
pH	<b>0.70</b>	-0.05
Al	-0.27	0.30
H+Al	-0.65	0.40
Ca	0.52	0.67

Mg	0.11	0.68
K	0.53	0.10
P	0.61	0.13
SB	0.40	<b>0.76</b>
CEC	0.03	<b>0.82</b>
V (%)	<b>0.78</b>	0.07
m (%)	-0.52	0.17
TOC	0.25	0.46
TN	-0.03	0.26
sand	<b>0.75</b>	-0.46
clay	<b>-0.70</b>	0.27
silt	-0.44	0.47
Cd	-0.47	-0.36
Co	0.11	-0.59
Cr	0.16	-0.27
Cu	-0.09	-0.14
Mo	-0.19	-0.23
Ni	0.30	-0.59
Pb	0.55	-0.07
V	-0.40	-0.29
Zn	-0.06	-0.44
Fe	-0.48	-0.21
Variance by component	Eigenvalue	% Total variance/Cumulative variance (%)
PC1	5.47	20.7 38.1
PC2	4.62	17.4

Bold marking >0.70

The surface layers (0–5 and 5–10 cm) showed a strong positive association with fertility-related attributes, such as pH, P, K, Ca, base saturation (V%), and sand content, all of which were correlated with the first principal component (PCA1), which explained 20.7% of the total variance (Fig. 3 and Table 4). This pattern reflects the typically higher fertility of surface soil layers, where greater concentrations of exchangeable bases are commonly observed. Also, along the PCA1 axis, a negative association was detected with potential acidity (H+Al), aluminum saturation (m%), sand, and silt—mainly concentrated in the 10–20 and 20–40 cm layers. This pattern suggests more acidic soil conditions in deeper layers, likely due to lower cation exchange capacity and the presence of clay and silt, which reduce nutrient mobility compared to the surface layers.

The deepest layer (40–60 cm) showed moderate negative correlations with several trace elements, including Cd, Co, Ni, and Zn, which were associated with the second

principal component (PCA2), accounting for 17.4% of the total variance (Fig. 3, Table 4).

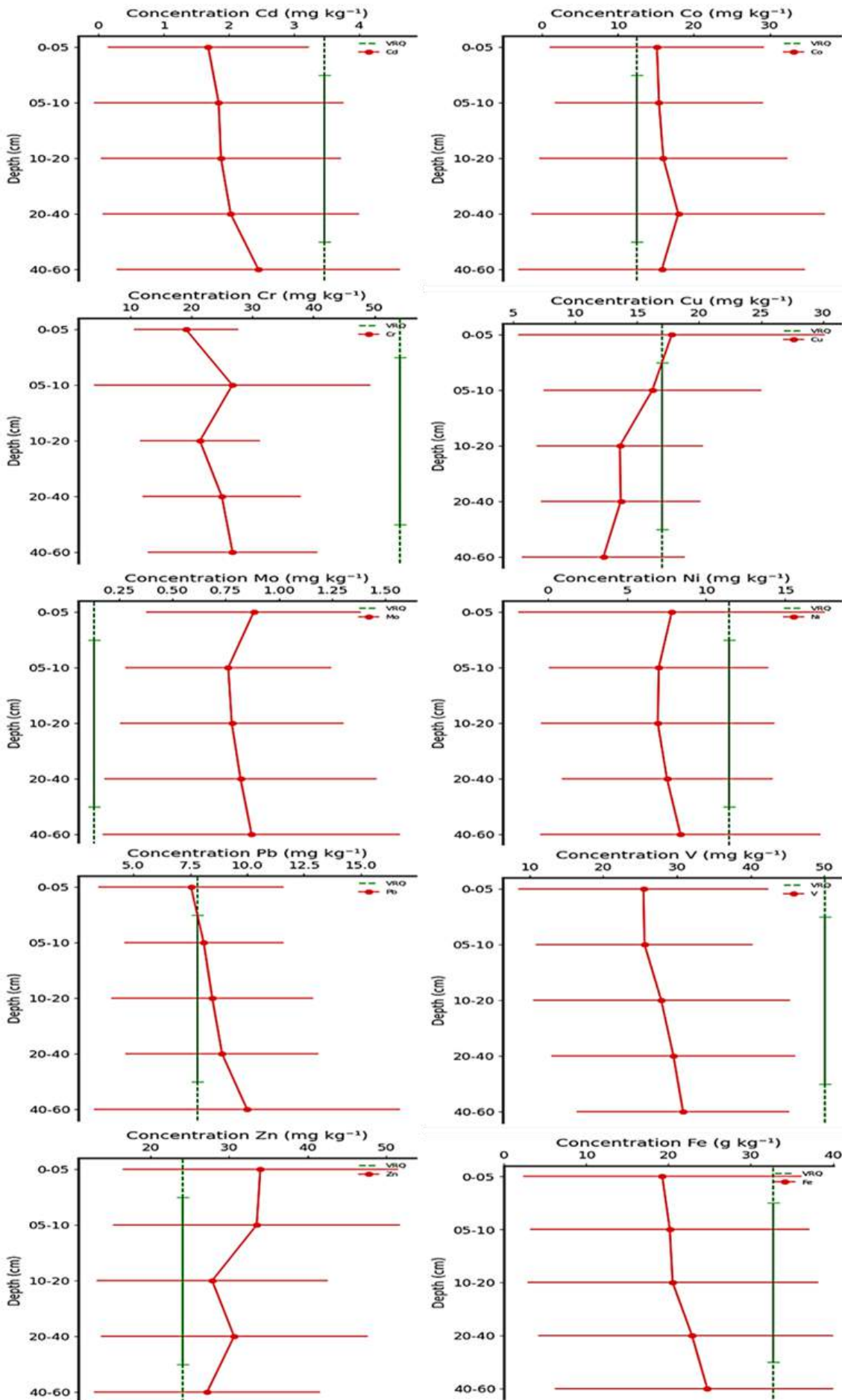
In contrast, cation exchange capacity (CEC) and sum of bases (SB) showed strong positive correlations with PCA2, suggesting an inverse relationship in this layer: increased chemical fertility is associated with decreased trace element concentrations.

### **5.3.5 Vertical distribution of trace elements in soil**

The vertical distribution of mean trace element concentrations across soil depths (Fig. 4) revealed similar patterns, with increasing concentrations at greater depths for Cd, Cr, Mo, Ni, Pb, V, and Fe. This trend may be explained by proximity to the parent material, where these elements naturally occur in higher concentrations (Kabata-Pendias, 2004). The geological composition of the region's soils (Fig. 1d) supports this observation, as they are derived from granite, diorite, and basalt—rock types known to be rich in Pb and Cd (Alloway, 2013). Sedimentary rocks also contribute to the presence of V, Pb, and Zn due to natural deposition processes (Kabata-Pendias; Mukherjee, 2007; Brito et al., 2020).

In the surface layers (0–5 and 5–10 cm), higher concentrations of Cu, Mo, Ni, and Zn were observed, likely influenced by environmental factors. One of the main mechanisms for the mobilization of these metals in soil is the reduction of pH (Schlutow et al., 2021). In acidic environments, metal complexes—especially  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ —become more soluble (Kabata-Pendias, 2011).

This surface accumulation of trace elements may also be related to the presence of organic matter. Root exudates and microbial activity release chelating compounds that bind to metals such as Cu, Mo, and Zn, increasing their bioavailability (Montiel-Rozas et al., 2016). Additionally, in poorly drained soils under reducing conditions can promote the dissolution of Fe and Mn oxides, releasing associated metal cations into the soil solution (Winkler et al., 2018). As the uppermost layer, this horizon is also more exposed to anthropogenic inputs, including intensive agricultural practices (Hu et al., 2024).



**Figure 4** - Vertical distribution of trace element concentrations and Soil Quality Reference Values (QRVs) in soils under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia, northeastern Brazil.

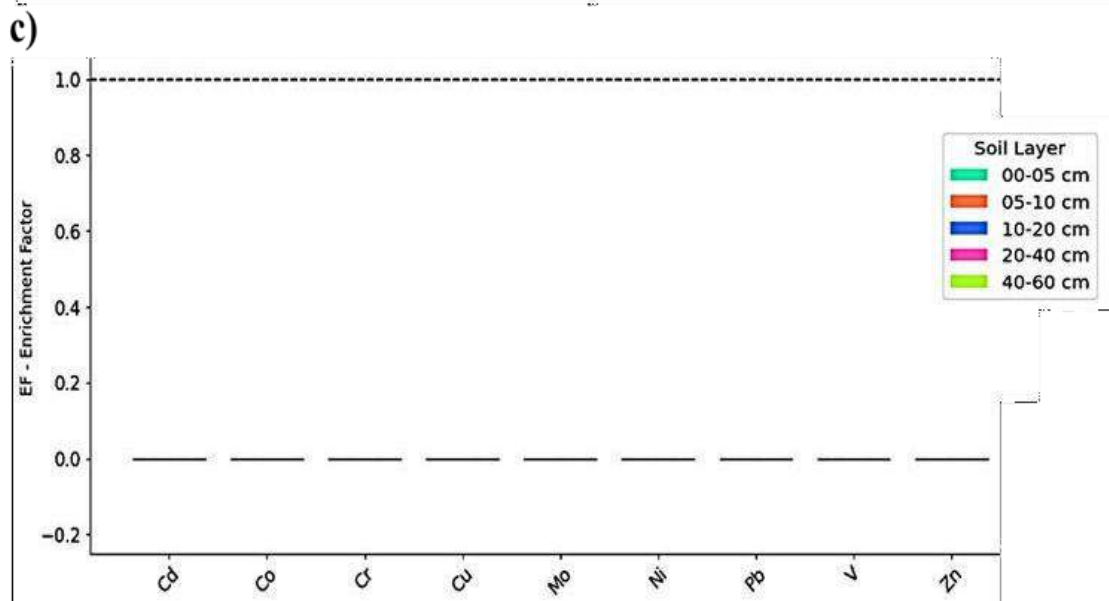
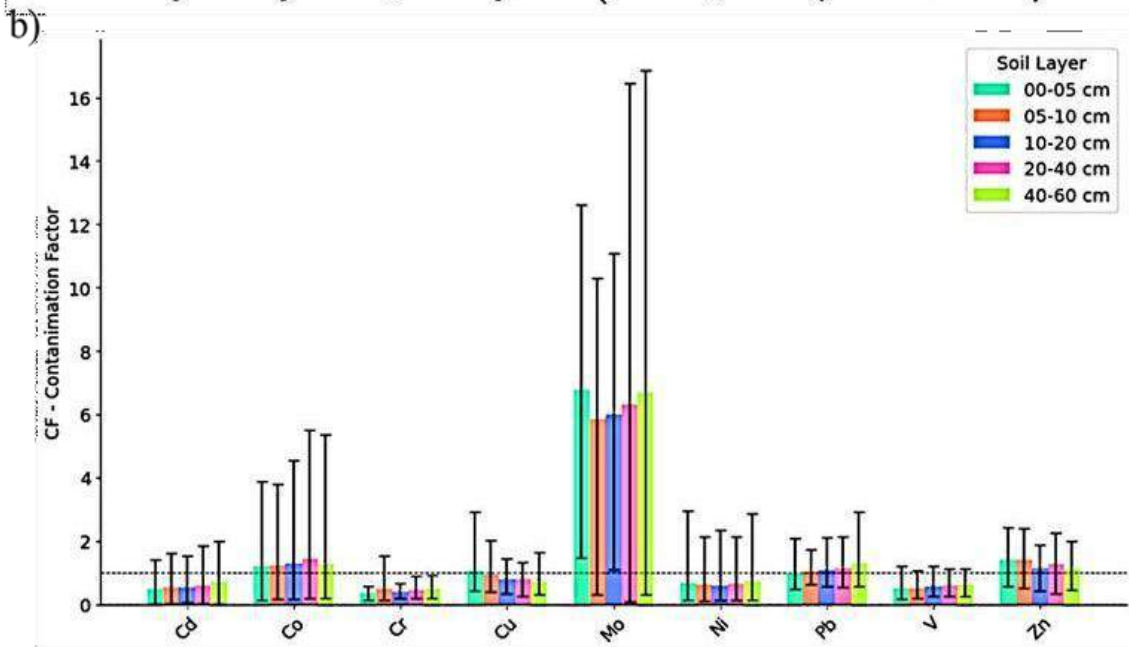
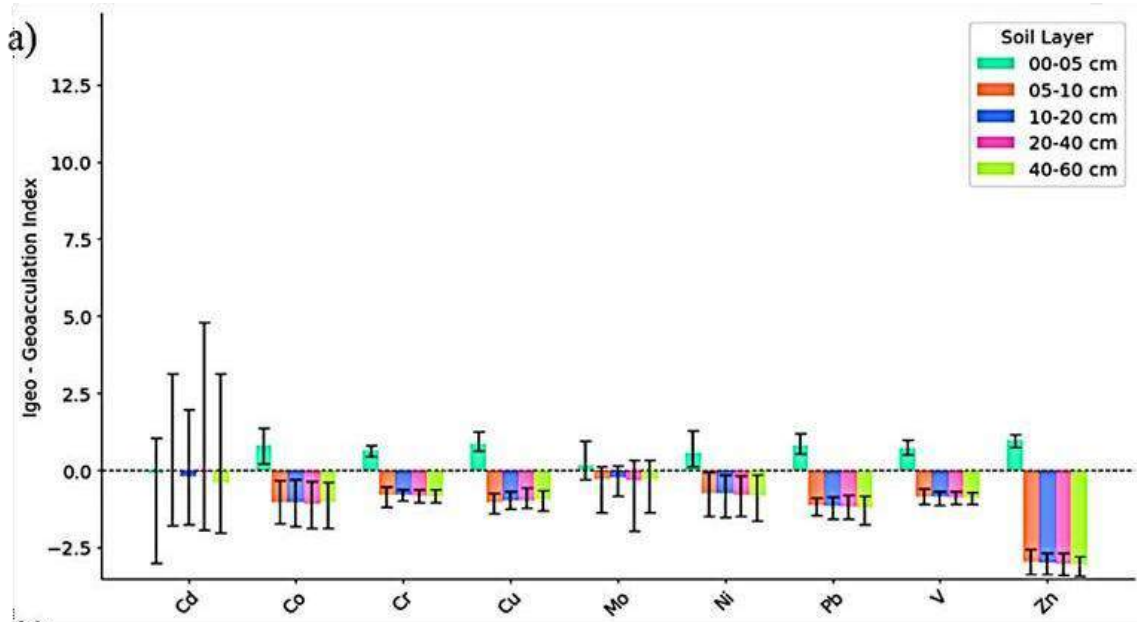
A significant portion of the trace elements exceeded the safe limits established by the soil Quality Reference Values (QRVs) defined for the cacao-growing region of Bahia (Bastos et al., 2025), ranked in ascending order ( $\text{mg kg}^{-1}$ ) as follows: Mo (0.13) < Cd (3.47) < Pb (7.81) < Ni (11.45) < Co (12.50) < Cu (16.99) < Zn (24.02) < V (50.12) < Cr(54.24) < Fe (32,740). Compared to these QRVs, the mean concentrations of Co, Cu, Mo, Pb, and Zn were approximately 1.3, 2.9, 6.3, 1.1, and 1.2 times higher, respectively (Fig.4).

These results highlight the need for continued monitoring and investigation in the region, as anthropogenic factors such as heavy traffic, mining, and agricultural practices are among the main contributors to the elevated levels of trace elements in soil (Zhang et al., 2023).

In contrast, Cd, Cr, Ni, V, and Fe exhibited concentrations below their respective QRVs, suggesting a relatively safe condition for these elements. However, even though Cd levels were below the regulatory threshold, monitoring Cd levels remains crucial—particularly in agricultural areas within ecologically sensitive regions such as Bahia's cocoa belt.

### **5.3.6 Accumulation indices for trace elements in soils**

The results obtained for the Geoaccumulation Index ( $I_{\text{geo}}$ ), calculated according to Eq. 1 and shown in Fig. 5a, indicated that, throughout the soil profile, most samples were classified as either unpolluted ( $I_{\text{geo}} < 0$ ) or unpolluted to moderately polluted ( $0 < I_{\text{geo}} < 1$ ), based on the classification scheme presented in Table 1. In the surface layer (0–5 cm), 88.89% of the samples indicated moderate pollution for elements such as Co, Cr, Cu, Mo, Ni, Pb, V, and Zn, with mean positive contamination factor values of 0.81, 0.66, 0.87, 0.19, 0.58, 0.81, 0.72, and 0.97, respectively. These results likely reflect a combination of natural sources or localized anthropogenic activities.



**Figure 5** - Individual values of (a) Geoaccumulation Index ( $I_{geo}$ ), (b) Contamination Factor (CF), and (c) Enrichment Factor (EF) for trace elements along the soil profile under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia, northeastern Brazil. The dotted line represents the threshold for considerable risk. Colored bars indicate individual values; black bars represent the standard error.

Based on the  $I_{geo}$  classification, only Cd at the 0–5 cm depth was classified as unpolluted, with an average value of 0.08, suggesting that soils under the cacao-cabruca agroforestry system exhibit low or no contamination by this element (Fig. 5a). This result may be attributed to the typical “Cabruca” management system, which largely excludes the use of agrochemicals, thereby reducing the presence or availability of Cd in the soil.

At greater depths (5–10, 10–20, 20–40, and 40–60 cm), negative  $I_{geo}$  values were recorded for all trace elements (Fig. 5a), classifying these soil layers as unpolluted. These findings indicate that the surface soil layer is more sensitive to anthropogenic impacts, as it is directly exposed to management practices that can alter the dynamics of trace elements in the soil.

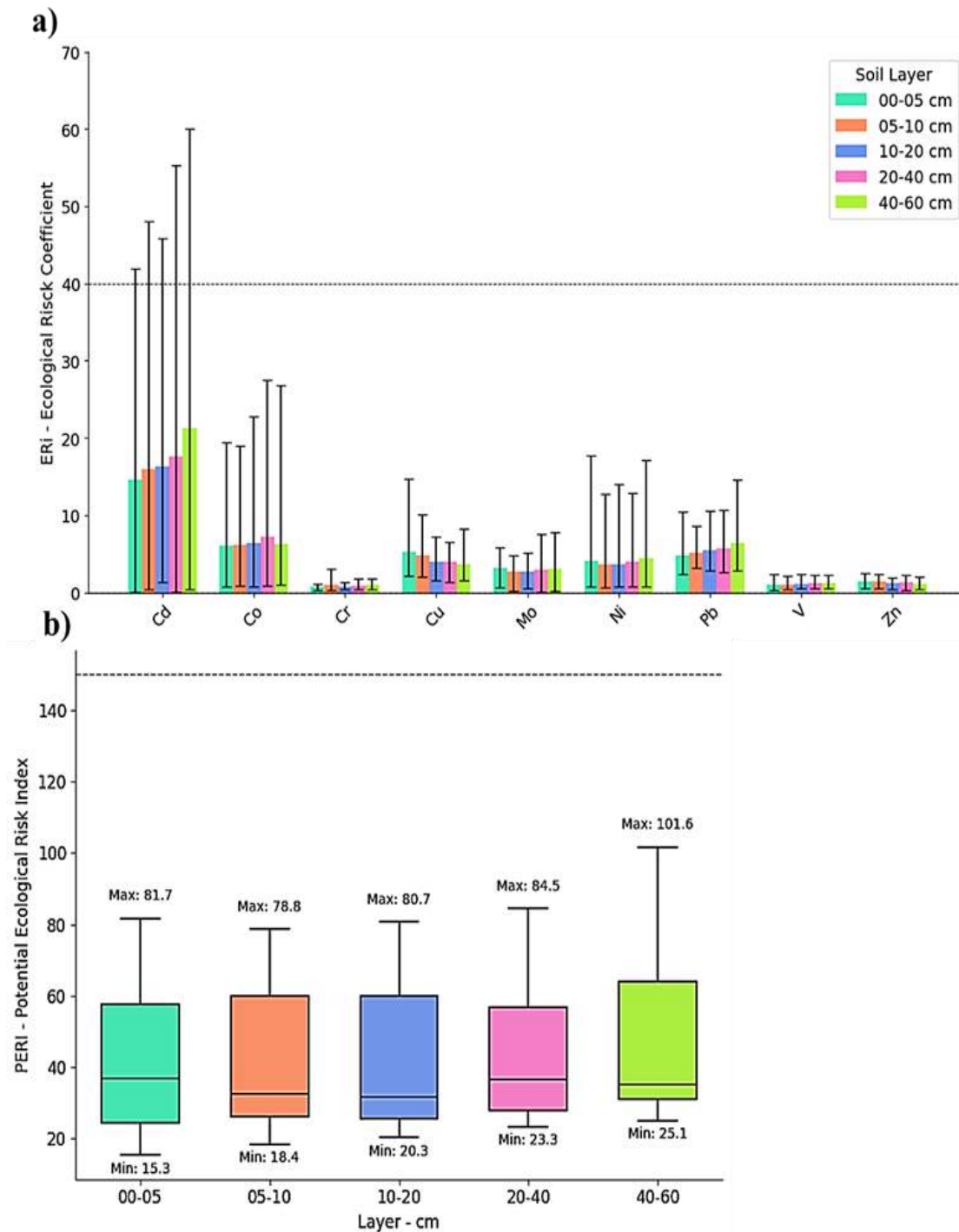
While the  $I_{geo}$  quantifies contamination levels by considering natural soil variability through a correction factor, the Contamination Factor (CF) provides a complementary, more direct, and objective estimate of contamination intensity based on the ratio between current element concentrations and their reference quality values. According to the contamination factor categories listed in Table 1, molybdenum (Mo) exhibited elevated contamination values throughout the soil profile (Fig. 5b), with CF values of 6.78 (0–5 cm), 5.86 (5–10 cm), 5.99 (10–20 cm), 6.31 (20–40 cm), and 6.70 (40–60 cm). This excess may be explained by the inappropriate use of soil amendments such as lime, which raises soil pH and can increase Mo bioavailability, favoring its accumulation (Li et al., 2019). Co and Zn showed low to moderate contamination levels, while Cd, Cr, Ni, and V were classified as uncontaminated.

The Enrichment Factor (EF) values were consistently zero for all trace elements throughout the soil profile (Fig. 5c), suggesting no anthropogenic enrichment and indicating that element concentrations reflect the natural geological background (Kowalska et al., 2016). This finding is consistent with Sutherland’s (2000) criteria, where  $EF \leq 1$  indicates natural levels and  $EF = 0$  corresponds to no external interference.

Overall, contamination assessment based on ecological indices ( $I_{geo}$ , CF, and EF) demonstrates that trace element contamination along the soil profile under the cacao-cabruca agroforestry system is moderate and limited to certain elements in the surface soil layer (0–5 cm), with no evidence of severe contamination. These results suggest that

the study area is free from high contamination levels and poses low environmental risks.

Risk assessment criteria for each metal ( $ER_i$ ), based on soil background values and toxicity coefficients, were evaluated according to Equation 4 and are presented in Fig. 6 and Table 4.



**Figure 6** - Values of (a) Individual Ecological Risk Index ( $ER_i$ ) and (b) Potential Ecological Risk Index (PERI) for trace elements along the soil profile in soils under the cacao-cabruca agroforestry system in the cacao-growing region of Bahia. The dotted line represents the threshold for considerable risk.

**Table 4.** Individual Ecological Risk Index ( $ER_i$ ) of trace elements in soils under the cacao- cabruca agroforestry system in the cacao-growing region of Bahia.

ERi class	$ER_i < 40$					$40 \leq ER_i < 80$					$80 \leq ER_i < 360$				
	0-5	5-10	10-20	20-40	40-60	0-5	5-10	10-20	20-40	40-60	0-5	5-10	10-20	20-40	40-60
Cd	90	80	80	80	80	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0
Co	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cr	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cu	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mo	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ni	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pb	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
V	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Zn	100	100	100	100	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

The categories  $80 \leq ER_i < 160$ ;  $160 \leq ER_i < 320$ ; and  $\geq 360$  were grouped into a single category:  $80 \leq ER_i < 360$ . According to the results obtained, nearly all  $ER_i$  values for trace elements in the cacao-cabruca agroforestry systems fell within the low ecological risk category, except for Cd. In the 0–5 cm layer, 90% of the samples were classified as low risk, while 10% exhibited moderate risk (Table 4). These findings highlight the elevated ecological risks associated with Cd contamination in agricultural areas, underscoring the need for targeted environmental management strategies to mitigate potential adverse effects on the cacao-cabruca agroforestry system.

Overall, the highest  $ER_i$  values among the analyzed trace elements were found for Cd (Fig. 6a, Table 4), primarily due to its relatively high Toxic Response Factor ( $Tr = 30$ ). When assessing individual trace element risks, the toxicity coefficient ( $Tr$ ) often plays a significant role in determining ecological risks (Zhang et al., 2022; Savic et al., 2025).

The ecological risk assessment based on the Potential Ecological Risk Index (PERI) revealed values ranging from 15.3 to 101.6 (Fig. 6b, Table 1), indicating that all sampled sites presented low ecological risk, with PERI values below 150 throughout the soil profile.

Taken together, these results show that a substantial portion of soils in the cacao-growing region of Bahia exhibited low levels of trace element contamination, as confirmed by established reference and quality values as well as soil environmental quality indices. This outcome reinforces the natural characteristics of the region's soils, indicating low anthropogenic influence and good environmental integrity in areas where Bahia's cacao is produced. The absence of significant enrichment of evaluated trace elements throughout the soil profile suggests that traditional agricultural practices, such as the

cacao-cabruca agroforestry systems, play a key role in maintaining soil quality.

## 5.4 CONCLUSION

The main findings emphasize that the mean concentrations of Co, Cu, Mo, Pb, and Zn in the study area's soils significantly exceeded the Soil Quality Reference Values (SQRVs) established for the cacao-growing region, displaying notably high levels. The spatial distribution of trace elements along the soil profile was largely governed by the parent material, with higher contents of some elements observed in the deeper soil layers.

Ecological risks based on  $ER_i$  indicated that trace elements Co, Cr, Cu, Mo, Ni, Pb, V, Zn, and Fe fell within the low pollution category across all evaluated layers, with 100% of samples classified as unpolluted. In contrast, Cd showed approximately 20% pollution proportion throughout the soil profile. The PERI confirmed the satisfactory quality of soils under the cacao-cabruca agroforestry system, as all layers were classified as low risk ( $PERI < 150$ ).

These results reinforce the importance of assessing concentrations, spatial distribution, and ecological risks associated with trace elements in cacao-cabruca agroforestry systems. Such approaches are vital for understanding the impacts of natural processes and human activities, while also informing public policy strategies aimed at mitigating trace element pollution in the cacao-growing region of Bahia.

## 5.5 REFERENCES

Adriano, D. C. 2001. Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals, Vol. 860. New York: Springer.

Ahmad, Z., Khan, S. M., Page, S. E., Balzter, H., Ullah, A., Ali, S., Mukhamezhanova, A. S. 2023. Environmental sustainability and resilience in a polluted ecosystem via phytoremediation of heavy metals and plant physiological adaptations. *Journal of Cleaner Production*, 385, 135733. <https://doi.org/10.1016/j.jclepro.2022.135733>

Ali, H., Khan, E., Sajad, M. A. 2013. Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, 91(7),869–881. <https://doi.org/10.1016/j.chemosphere.2013.01.075>

Alloway, B.J. 2013. *Heavy Metals in Soils*. Springer. <https://doi.org/10.1007/978-94-007-4470-7>

Alves, P.N., Cardoso, K.M., Nascimento, C.W.A., Barros, J.S., Sena, A.F.S., Morais, P.G.C., Saraiva, P.C., Escobar, M.E.O., Cunha, K.P.V., Boechat, C.L., 2024.

Heavy metals in soils derived from sedimentary rocks of the Gurgueia River watershed, Northeast, Brazil: background values, distribution and ecological risk assessment. *Environ. Geochem. Health* 46, 438. <https://doi.org/10.1007/s10653-024-02216-8>

Baldock, J. A., Sanderman, J., Macdonald, L. M., Puccini, A., Hawke, B., Szarvas, S., McGowan, J., 2013. Quantification of soil organic carbon allocation for biologically significant fractions. *Soil Research*, 51(8), 561-576. <https://doi.org/10.1071/SR12374>

Baltas, H., Sirin, M., Gökbayrak, E., & Ozcelik, A. E. 2020. A case study on pollution and a human health risk assessment of heavy metals in agricultural soils around Sinop province, Turkey. *Chemosphere*, 241, 125015. <https://doi.org/10.1016/j.chemosphere.2019.125015>

Bastos, T. R.S., Medeiros, J. C., do Nascimento, C. W. A., Cardoso, K. M., Alves, P. N., Morais, P. G. C., Boechat, C. L. 2025. Geochemical background of some trace elements in Atlantic Forest soils in a cacao-producing region and its implications for human health and food safety. *Chemosphere*, 377, 144341. <https://doi.org/10.1016/j.chemosphere.2025.144341>

Barkett, M. O., Akün, E. 2018. Heavy metal contents of contaminated soils and ecological risk assessment in abandoned copper mine harbor in Yedidalga, Northern Cyprus. *Environmental Earth Sciences*, 77(10), 378. <https://doi.org/10.1007/s12665-018-7556-6>

Boechat, C.L., Duarte, L.S.L., Sena, A.F.S., Nascimento, C.W.A., Silva, Y.J.A.B., Brito, A.C. C., Saraiva, P.C., 2020. Background concentrations and quality reference values for potentially toxic elements in soils of Piauí state, Brazil. *Environ. Monit. Assess.* 192, 723. <https://doi.org/10.1007/s10661-020-08656-w>

Bolan, N. S, Makino, T., Kunhikrishnan, A., Kim, P.J., Ishikawa, S., Murakami, M., Kirkham, M. B., 2013. Cadmium contamination and its risk management in rice ecosystems. *Advances in agronomy*, 119, 183-273. <https://doi.org/10.1016/B978-0-12-407247-3.00004-4>

Brasil. Ministério da Agricultura e Pecuária – MAPA. 2024. Produção e exportação de cacau e derivados: relatório anual 2024. Brasília: MAPA. Disponível em <https://www.gov.br/agricultura>.

Cai, L. M., Wang, Q. S., Wen, H. H., Luo, J., Wang, S., 2019. Heavy metals in agricultural soils from a typical township in Guangdong Province, China: Occurrences and spatial distribution. *Ecotoxicology and environmental safety*, 168, 184-191. <https://doi.org/10.1016/j.ecoenv.2018.10.092>

Cardoso, K.M., Boechat, C.L., Nascimento, C.W.A., Escobar, M.E.O., Silva, D.G., Lins, S.A. S., Morais, P.G.C., 2023. Watershed-scale assessment of the environmental values of potentially toxic soil elements of the Caatinga and Atlantic Forest ecotone in Brazil. *Chemosphere* 338, 139394. <https://doi.org/10.1016/j.chemosphere.2023.139394>

Cardoso, K.M., Nascimento, C.W.A., Lins, S.A.S., Nascimento, C.C., Oliveira, R.L., Silva, D.G., Morais, P.G.C., Boechat, C.L., 2024. Assessing ecological risks and spatial distribution of potentially toxic elements in soils from anthropized environments in a

watershed at the caatinga-Atlantic Forest ecotone in Brazil. *Environ. Res.* 249, 118423. <https://doi.org/10.1016/j.envres.2024.118423>

Chen D., Shengfei Y., Zhiyang J., Zhirui W.g, Zhanbin W., Hui T., 2023. Spatial distribution, ecological risk and health risk assessment of heavy metals in agricultural soil from Ankang basin, Shaanxi Province, *Heliyon*, Vol. 9, Issue 12. <https://doi.org/10.1016/j.heliyon.2023.e22580>

Conselho Nacional do Meio Ambiente - CONAMA, 2009. Resolução N. 420 de 28 de fevereiro de 2009. from. [http://www.mma.gov.br/port/conama/res/res09/res\\_42009.pdf](http://www.mma.gov.br/port/conama/res/res09/res_42009.pdf). (Accessed 26 February 2025).

Ejaz, U., Khan, SM, Khalid, N., Ahmad, Z., Jehangir, S., Fatima Rizvi, Z., Raposo, A., 2023. Heavy Metal Detoxification: A Multifaceted Study of Heavy Metal Toxicity Tolerance Strategies in Plants. *Frontiers in plant science*, 14, 1154571. <https://doi.org/10.3389/fpls.2023.1154571>

Fei, X., Xiao, R., Christakos, G., Langousis, A., Ren, Z., Tian, Y., Lv, X., 2019. Comprehensive assessment and source apportionment of heavy metals in Shanghai agricultural soils with different fertility levels. *Ecological Indicators*, 106, 105508. <https://doi.org/10.1016/j.ecolond.2019.105508>

Gallardo, A., Paramá, R., 2007. Spatial variability of soil elements in two plant communities of NW Spain. *Geoderma*, 139(1-2), 199-208. <https://doi.org/10.1016/j.geoderma.2007.01.022>

Hakanson L., 1980 An ecological risk index for the control of aquatic pollution. A sedimentological approach. *Water research*. January 1, 1980; 14(8):975-1001. [https://doi.org/10.1016/0043-1354\(80\)90143-8](https://doi.org/10.1016/0043-1354(80)90143-8)

Hou, D., O'Connor, D., Igalavithana, A. D., Alessi, D. S., Luo, J., Tsang, D. C., Ok, Y. S., 2020. Metal contamination and bioremediation of agricultural soils for food safety and sustainability. *Nature Reviews Earth & Environment*, 1(7), 366-381. <https://doi.org/10.1038/s43017-020-0061-y>

Hu, J., Wang, Z., Williams, G. D. Z., Dwyer, G. S., Gatiboni, L., Duckworth, O. W., Vengosh, A., 2024. Evidence for the accumulation of toxic metal(loid)s in agricultural soils impacted from long-term application of phosphate fertilizer. *Science of Total Environment*, 907, 167863. <https://doi.org/10.1016/j.scitotenv.2023.167863>

Huang, J. H, Liu, W. C, Zeng, G. M, Li, F., Huang, X. L, Gu, Y. L, Wan, J., 2016. An exploration of the spatial assessment of the risk to human health of toxic soil metals under different land uses using simulation of sequential indicators. *Ecotoxicology and Environmental Safety*, 129, 199-209. <https://doi.org/10.1016/j.ecoenv.2016.03.029>

Huang, B., Li, Z., Li, D., Yuan, Z., Chen, Z., Huang, J., 2017. Heavy metal distribution characteristics (loid) in aggregates of different size fractions along the soil profile of contaminated rice. *Environmental Science and Pollution Research*, 24, 23939-23952. <https://doi.org/10.1007/s11356-017-0012-4>

Instituto Brasileiro de Geografia e Estatística – IBGE. 2024. Levantamento Sistemático da Produção Agrícola – Área plantada, área colhida e produção por safra. Available at IBGE (accessed on 5 February 2025).

Islam, M. S., Ahmed, M. K., Habibullah-Al-Mamun, M., Masunaga, S., 2015. Assessment of trace metals in fish species of urban rivers in Bangladesh and health implications. *Environmental toxicology and pharmacology*, 39(1), 347-357. <https://doi.org/10.1016/j.etap.2014.12.009>

Kabata-Pendias, A., 2000. Trace elements in soils and plants. CRC press. <https://doi.org/10.1201/9781420039900>

Kabata-Pendias, A., 2004. Trace Elements in Soils and Plants. CRC Press.

Kabata-Pendias, A., 2011. Trace Elements in Soils and Plants. (4th ed.). CRC Press.

Khan, M. A., Khan, S., Khan, A., Alam, M., 2015. Soil contamination with cadmium, consequences and remediation using organic amendments. *Science of the Total Environment*, 601–602, 1591–1605. <https://doi.org/10.1016/j.scitotenv.2017.06.030>

Koppen, W., 1948. *Climatologia: com um estúdio de los climas de la tierra*. Laboratory of Climatology.

Kowalska, J., Mazurek, R., Gąsiorek, M., Setlak, M., Zaleski, T., Waroszewski, J., 2016. Soil pollution indices conditioned by medieval metallurgical activity—A case study from Krakow (Poland). *Environmental Pollution*, 218, 1023-1036. <https://doi.org/10.1016/j.envpol.2016.08.053>.

Li, Y., Qu, X., Zhang, M., Peng, W., Yu, Y., Gao, B., 2018. Anthropogenic impact and ecological risk assessment of thallium and cobalt in Poyang Lake using the geochemical baseline. *Water*, 10(11), 1703. <https://doi.org/10.3390/w10111703>.

Li, Y., Cui, S., Chang, S. X et al., 2019. The effects of liming on soil pH and crop productivity depend on the type of limestone material, the method and rate of application, and the crop species: a global meta-analysis. *J Soils Sediments* 19, 1393– 1406. <https://doi.org/10.1007/s11368-018-2120-2>

Li, J., Li, X., Tai, X. S. et al., 2025. Machine learning-assisted source identification and probabilistic evaluation of ecological and health risks of heavy metals (loids) in urban park soils. *Sci Rep.* 15, 17451. <https://doi.org/10.1038/s41598-025-02307-1>

Lian, M., Wang, J., Sun, L., Xu, Z., Tang, J., Yan, J., Zeng, X., 2019. Profiles and potential health risks of heavy metals in soil and crops from the watershed of Xi River in Northeast China. *Ecotoxicology and environmental safety*, 169, 442-448. <https://doi.org/10.1016/j.ecoenv.2018.11.046>

Lian, Z., Zhao, X., Gu, X., Li, X., Luan, M., & Yu, M., 2022. Presence, sources, and risk assessment of heavy metals in the upland soils of northern China using Monte Carlo simulation. *Ecotoxicology and Environmental Safety*, 230, 113154. <https://doi.org/10.1016/j.ecoenv.2021.113154>

Liu, P., Hu, W., Tian, K., Huang, B., Zhao, Y., Wang, X., Khim, J. S., 2020. Accumulation and ecological risk of heavy metals in soils along the coastal areas of the Bohai Sea and the Yellow Sea: A comparative study of China and South Korea. *Environment international*, 137, 105519. <https://doi.org/10.1016/j.envint.2020.105519>

Mansour, S. A., 2014. Heavy metal contamination as a global problem and the need for prevention/reduction measurements. *Practical food safety: contemporary issues and future directions*, 257-280. <https://doi.org/10.1002/9781118474563.ch14>

Montiel-Rozas M.M, Madejón E, Madejón P., 2016. Effect of heavy metals and organic matter on root exudates (low molecular weight organic acids) of herbaceous species: An assessment in sand and soil conditions under different levels of contamination. *Environ Pollut. Sep*; 216:273-281. <https://doi.org/10.1016/j.envpol.2016.05.080>

Muller, G., 1969. Geoaccumulation index in rhine river sediments. *Geo Journal*, 2(3), 108-118. SID. <https://sid.ir/paper/618491/en>.

N'guessan, Y. M., Probst, J. L., Bur, T., Probst, A., 2009. Trace elements in stream bed sediments from agricultural catchments (Gascogne region, SW France): where do they come from? *Science of the total environment*, 407(8), 2939-2952. <https://doi.org/10.1016/j.scitotenv.2008.12.047>

National Institute of Standards and Technology – NIST, 2002. Standard reference materials-SRM 2709, 2710 and 2711 from. <https://www-s.nist.gov/srmors/certificates/archives/2710.pdf>. (Accessed 10 April 2025).

Oliveira, E.P., McNaughton, N.J., Armstrong, R., 2010. Mesoarchean to Palaeoproterozoic Growth of the Northern Segment of the Itabuna-Salvador-Curaçá Orogen, Sao Francisco Craton, Brazil, vol. 338, pp. 263–286. <https://doi.org/10.1144/SP338.13>

Python Software Foundation. Python: A dynamic, open-source programming language. Version 3.11, 2024 Available at: <https://www.python.org>.

Rajendran, S., Priya, T. A. K, Khoo, K. S, Hoang, T. K, Ng, H. S, Munawaroh, H. S. H., Show, P. L., 2022. A critical review of several remediation approaches for removing heavy metal contaminants from contaminated soils. *Chemosphere*, 287, 132369. <https://doi.org/10.1016/j.chemosphere.2021.132369>

Raij, B., Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. Análise química para avaliação da fertilidade de solos tropicais. IAC. Rutkowski, P., Diatta, J., Konatowska, M., Andrzejewska, A., Tyburski, Ł., Przybylski, P., 2020. Geochemical referencing of natural forest contamination in Poland. *Forests*, 11(2), 157. <https://doi.org/10.3390/f11020157>

Saha, A., Gupta, B. S, Patidar, S., Hernández-Martínez, J. L, Martín-Romero, F., Meza-Figueroa, D., Martínez-Villegas, N. 2024. A comprehensive study of source apportionment, spatial distribution, and health risks assessment of heavy metal(loid)s in the surface soils of a semi-arid mining region in Matehuala, Mexico, *Environmental Research*, Vol 260, 119619. <https://doi.org/10.1016/j.envres.2024.119619>

Sarwar, N., Imran, M., Shaheen, MR, Ishaque, W., Kamran, MA, Matloob, A., 2017. Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. *Chemosphere*, 171,710–721. <https://doi:10.1016/j.chemosphere.2016.12.116>

Savic, R., Stajic, M., Kostesic, E., Antic, S., Horvatinec, J., Zemunac, R., Ondrasek, G., 2025. Heavy metal contamination and ecological risk assessment in drainage channel sediments from urban and agricultural areas. *Journal of Environmental Management*, 389, 126221. <https://doi.org/10.1016/j.jenvman.2025.126221>

Schlutow, A., Schröder, W., Scheuschner, T., 2021. Assessing the relevance of atmospheric heavy metal deposition with regard to ecosystem integrity and human health in Germany. *Environmental Science Eur* 33, 7. <https://doi.org/10.1186/s12302-020-00391-w>

Šimanský, V., Jonczak, J., 2020. Aluminum and iron oxides affect the soil structure in a long-term mineral fertilized soil. *J Soil Sediments* 20, 2008–2018. <https://doi.org/10.1007/s11368-019-02556-4>

Soil Survey Staff., 2014. *Keys to Soil Taxonomy*, twelfth ed. USDA - Natural Resources Conservation Service, Washington, DC.

Souza, H. N., de Goede, R. G., Brussaard, L., Cardoso, I. M., Duarte, E. M., Fernandes, R. B., Pulleman, M. M., 2019. Protective shade, tree diversity and soil properties in cacao agroforestry systems in southern Bahia, Brazil. *Agriculture, Ecosystems & Environment*, 266, 17–25. <https://doi.org/10.1016/j.agee.2011.11.007>

Sutherland, R. A., 2000. Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. *Environmental Geology*, 39(6), 611–627. <https://doi.org/10.1007/s002540050473>

Teixeira, P.C. et al., 2017. *Manual de métodos de análise de solo*. 3. ed. rev. e ampl. – Brasília, DF.

United States Environmental Protection Agency – USEPA, 2007. Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils. Revision 1 from <https://www.epa.gov/sites/default/files/2015-12/documents/3051a.pdf>. (Accessed 12 March, 2025).

Wang, Y., Yang, L., Kong, L., Liu, E., Wang, L., Zhu, J., 2015. Spatial distribution, ecological risk assessment and source identification for heavy metals in surface sediments from Dongping Lake, Shandong, East China. *Catena*, 125, 200–205. <https://doi.org/10.1016/j.catena.2014.10.023>

Wang, X., Ai, S., & Liao, H., 2023. Deciphering interactions between phosphorus status and toxic metal exposure in plants and rhizospheres to improve crops reared on acid soil. *Cells*, 12(3), 441. <https://doi.org/10.3390/cells12030441>

Winkler, P., Kaiser, K., Thompson, A., Kalbitz, K., Fiedler, S., Jahn, R., 2018. Contrasting evolution of iron phase composition in soils exposed to redox fluctuations. *Geochimica et Cosmochimica Acta*, 235, 89–102.

<https://doi.org/10.1016/j.gca.2018.05.019>

Zhang, H., Jiang, Y., Ding, M., Xie, Z., 2017. Level, source identification, and risk analysis of heavy metal in surface sediments from river-lake ecosystems in the Poyang Lake, China. *Environmental Science and Pollution Research*, 24, 21902-21916. <https://doi.org/10.1007/s11356-017-9855-y>

Zhang, T., Wang, P., Wang, M., Liu, J., Gong, L., Xia, S., 2023. Spatial distribution, source identification and risk assessment of heavy metals in riparian soils of the Tibetan plateau. *Environmental Research*, 237, 116977. <https://doi.org/10.1016/j.envres.2023.116977>

Zhao, S., Feng, C., Yang, Y., Niu, J., Shen, Z., 2012. Risk assessment of sedimentary metals in the Yangtze Estuary: New evidence of the relationships between two typical index methods. *Journal of hazardous materials*, 241, 164-172. <https://doi.org/10.1016/j.jhazmat.2012.09.023>

Zhu, X. F., Shen, R. F., 2024. Towards sustainable use of acidic soils: Deciphering aluminum-resistant mechanisms in plants. *Fundamental Research*, 4(6), 1533-1541. <https://doi.org/10.1016/j.fmre.2023.03.004>

Zovko, Monika., Romic, Marija. 2011. Soil Contamination by Trace Metals: Geochemical Behaviour as an Element of Risk Assessment. 437-456. <https://doi.org/10.5772/25448>

## 6. FINAL CONSIDERATIONS

Scientific research on cacao plays a strategic role not only for strengthening agricultural production but also in advancing our understanding of ecological processes in tropical ecosystems. Cacao-based systems, especially agroforestry, are natural laboratories for investigating soil-plant-atmosphere relationships, offering valuable insights into how sustainable agricultural practices influence the carbon cycle and the dynamics of heavy metals in soils.

Based on the results obtained, the proposed objectives of this research were achieved, demonstrating the environmental importance of the cacao-growing region of Bahia, especially the cacao-cabruca agroforestry system, in regulating soil carbon dynamics and mitigating trace elements pollution.

A considerable accumulation of carbon was observed along the soil profile (0- 60 cm) in the cacao-cabruca agroforestry systems, particularly in the deeper layers. Unlike the surface layers, deeper soil fractions act as stable reservoirs with limited human interference, thereby enhancing carbon storage and contributing to climate change mitigation, which ensures the sustainability of the agricultural system.

Variations in trace element concentrations were also identified in natural soils of the cacao region of Bahia, as revealed by the determination of reference values and soil quality indices (VRQ). Environmental complexity, such as heterogeneous soil classes, geology, topographic changes and climate, favored the mobility and availability of trace elements in the soil. For this reason, smaller-scale studies enable more accurate environmental monitoring.

Although ecological risk analyses indicated that anthropogenic activities contribute to increased availability of some trace elements, the study classified the region within the low to moderate risk category. However, further research is required to determine the origin and specific sources of each element, particularly Cd, which poses an individual ecological risk to the region.

Systematic monitoring of soil quality indicators provides robust evidence that allows for the validation and refinement of theoretical models of soil functioning. This process not only strengthens existing scientific approaches but also supports the development of new methodologies for sustainability assessment.

Furthermore, it establishes a unique and comparable scientific foundation that can guide analyses across diverse environmental contexts and inform public policies and

management strategies. Specifically, in tropical agroecosystems, this approach enables global-scale comparisons, highlighting both the challenges and opportunities these systems face in relation to climate change, biodiversity conservation, and carbon sequestration.

Ultimately, continuous monitoring of soil health reinforces the transition towards more resilient and sustainable agricultural practices, with direct impacts on food security, emission mitigation, and the maintenance of essential ecosystem services.

## 7. ANNEX

### ANNEX A

Chemosphere 377 (2025) 144341

Contents lists available at ScienceDirect

**Chemosphere**

journal homepage: [www.elsevier.com/locate/chemosphere](http://www.elsevier.com/locate/chemosphere)

Check for updates

### Geochemical background of some trace elements in Atlantic Forest soils in a cocoa-producing region and its implications for human health and food safety

Tatiana Reis dos Santos Bastos<sup>a</sup>, João Carlos Medeiros<sup>b</sup>,  
Clistenes Williams Araújo do Nascimento<sup>c</sup>, Kaique Mesquita Cardoso<sup>a,d</sup>,  
Paula Nascimento Alves<sup>c</sup>, Pâmella Graziely Carvalho Moraes<sup>c</sup>, Geison dos Santos Pereira<sup>b</sup>,  
Ana Luísa Leite Pereira<sup>a</sup>, Maria Eugênia Ortiz Escobar<sup>e</sup>, Cácio Luiz Boechat<sup>a,e,f</sup>

<sup>a</sup> State University of Southwest Bahia (UESB), Graduate Program in Agronomy, Vitória da Conquista, Bahia, 45063-900, Brazil  
<sup>b</sup> Federal University of Southern Bahia (UFSEB), Itabuna, Bahia, 45600-923, Brazil  
<sup>c</sup> Federal Rural University of Pernambuco (URPE), Recife, Pernambuco, 52171-900, Brazil  
<sup>d</sup> Federal Institute of Education, Science and Technology of Northern Minas Gerais (IFNMG), Araxá, Minas Gerais, 39600-000, Brazil  
<sup>e</sup> Federal University of Piauí (UFPI), Campus Professor Condebaldo Silva, Rodovia Bom Jesus - Viana, S/n, Planalto Hortoatense, Bom Jesus, Piauí, 64900-000, Brazil  
<sup>f</sup> Federal University of Ceará (UFCE), Fortaleza, Ceará, 60435780, Brazil

#### HIGHLIGHTS

- A high concentration of naturally occurring cadmium and barium was reported in certain areas.
- Concentrations strongly correlate with parent material and pedogenic processes.
- Quality Reference Values in Atlantic Forest soils were proposed at the 75th percentile.
- These values will serve as a basis for monitoring and enforcement in the region.

#### GRAPHICAL ABSTRACT

Geochemical background of some trace elements in Atlantic Forest soils in a cocoa-producing region and its implications for human health and food safety

Map of Brazil showing the location of Bahia state. Map of Bahia state showing the location of the Atlantic Forest region. Map of the Atlantic Forest region showing 28 sampling points. Cross-section of soil layers (0-10 cm and 10-50 cm). Analytical methods: Data availability (Statistical analysis: ANOVA, PCA, etc.), Determination of QRVs, Parent concentrations of Cd, Pb, and Ba, and Growth/Quality (Fertilis, FDS).